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(90-day results on p. 682)

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TOXICOLOGY AND APPLIED PHARMACOLOGY 17, 679-690 (1970)

Toxicity of Paraquat to Rats and Its Effect on Rat Lungs

R. D. KIMBROUGH AND T. B. GAINES

Division of Pesticide Chemistry and Toxicology, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, 4770 Buford Highway, Chamblee, Georgia 30341

Received November 10, 1969

Toxicity of Paraquat to Rats and Its Effect on Rat Lungs. KIMBROUGH, R. D., and GAINES, T. B. (1970). *Toxicol. Appl. Pharmacol.* 17, 679-690. Acute and subacute toxicity studies were conducted with paraquat (1,1'-dimethyl-4,4'-dipyridylium dimethylsulfate) in adult Sherman rats. The single-dose oral LD50 was 100 mg/kg in male rats and 110 mg/kg in females. The 90-dose oral LD50 in female rats was 21 mg/kg, giving a chronicity factor of 5.2. The acute dermal LD50 was 80 mg/kg in male rats and 90 mg/kg in females. Studies of lung tissue with the electron microscope after a single oral dose indicate that the first discernible changes are pulmonary edema, swelling of the epithelium, an increase in collagen, and an effect on the ribosomes of the membranous pneumocytes. Light microscopic studies of lungs affected by paraquat after po and iv administration or local instillation showed intra-alveolar hemorrhage, edema, extensive fibrosis, and changes in the epithelium. Local instillation of 0.05 mg/kg of paraquat into lung and 0.8 mg/kg into skeletal muscle caused local fibrosis.

The dipyridylium compound paraquat (1,1'-dimethyl-4,4'-dipyridylium dimethylsulfate) is widely used as a weed killer (Thomson, 1967). In their studies on its toxicity in rats, mice, dogs, and rabbits, Clark *et al.* (1966) found that paraquat had a specific effect on the lungs of these animals. The intake of paraquat was followed by fibroblastic and epithelial proliferation, leading to complete consolidation. Several cases of human poisoning have also been reported (Bullivant, 1966; Almog and Tal, 1967; Campbell, 1968; Fennelly *et al.*, 1968; Matthew *et al.*, 1968; McKean, 1968; Oreopoulos *et al.*, 1968). The lungs were similarly affected in these cases, and respiratory failure was the cause of death in most instances. In acute or chronic poisoning with paraquat, the lung lesions may not become apparent until some time after dosing. Dar'el and Gage (1970) reported that following a single oral dose most of the compound is excreted in the urine and feces within about 2 days. Because of its rapid excretion and the delayed effect on the lungs, Clark *et al.* (1966) assumed that paraquat initiates irreversible changes in the lungs that do not become apparent until later. Manktelow (1967) found that the pulmonary surfactant was severely reduced or absent in mice that had been given paraquat intravenously. Recently Calderbank (1968), Akhaven and Linscott (1968), and Conning *et al.* (1969) reviewed various actions, properties, and effects of dipyridylium herbicides.

This paper reports the results of our studies of the toxicity of paraquat and its local effect on the lungs of Sherman rats.

METHODS

Adult Sherman rats 3–6 months old were used. They were caged individually and weighed at weekly intervals. The paraquat[1] used was a formulation of the concentrate containing 2 lbs of the paraquat cation per gallon of water. The concentrate was diluted in water in appropriate concentrations for oral, dermal, or intravenous dosing and for instillation into the lungs and skeletal muscle. Dosage or dietary levels were calculated on the basis of the paraquat cation content.

Lung tissue for light microscopic study was fixed in buffered 10% formalin and stained with hematoxylin and eosin. Lung tissue for electron microscopic study was obtained immediately after decapitation of the animals. The tissue was cut into 1–2 mm pieces and fixed in chilled 0.1 M phosphate-buffered 5% glutaraldehyde, postfixed in chilled buffered 1% osmium tetraoxide, embedded in Maraglas following dehydration, and usually stained with lead citrate and uranyl acetate. Sections were cut with glass knives and examined with the Philips EM-300 electron microscope.

Acute toxicity studies. The 1-dose oral LD50 values were determined in 50 male rats weighing 290–554 g and in 40 females weighing 208–290 g. The acute dermal LD50 values were determined in 50 male rats weighing 296–538 g and in 40 females weighing 216–290 g. The paraquat concentrations in water were varied, and the volumes given to the rats were held constant at 0.005 ml/g of body weight by stomach tube and 0.0016 ml/g by dermal application. Otherwise, the methods of conducting the LD50 studies were the same as those described by Gaines (1960). The rats were held for observation for a minimum of 15 days after treatment. Autopsies were not performed on the rats that survived.

The 90-day LD50 was determined according to methods described by Hayes (1967). Five groups of rats, each consisting of 10 females weighing from 221 to 357 g, were fed paraquat at dietary levels of 300, 400, 500, 650, and 700 ppm, respectively, for 90 days. The diets were prepared by mixing a predetermined amount of paraquat concentrate and an equal amount of 95% ethyl alcohol with 100 g of corn starch, using a spatula. The alcohol served to reduce the surface tension of the paraquat concentrate. The corn starch-paraquat was mixed with 350 g of ground laboratory chow, and then mixed with the proper quantity of ground chow to give the most concentrated diet used. Diets containing lower concentrations of paraquat were prepared by further dilution a portion of the next higher concentration with ground chow. All diets were mixed with a mechanical mixer. The food consumption was recorded daily during weeks 1, 2, 7, and 12 to determine the amount of paraquat consumed in mg/kg day. To allow time for possible death of the surviving rats, they were held for 2 weeks after termination of exposure. The lungs of all rats that died and of all survivors were examined grossly and microscopically.

The LD50 values were calculated by the method of Litchfield and Wilcoxon (1949).

To provide lung tissue at specified intervals following a single dose, 4 male rats were given 400 mg/kg of paraquat and 2 other males were given 150 mg/kg by stomach tube. At both 1 and 4 hr after dosing, 2 of the rats given 400 mg/kg and 1 control were killed by decapitation. The rats given 150 mg/kg and 1 control were killed 4 hr after dosing. Lung tissue from each rat was examined by the light and the electron microscope.

[1] Supplied through the courtesy of the Chevron Chemical Company.

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Dosages of 3.0 and 6.0 mg/kg of paraquat were given to 5 male rats each by tail vein injection to test the effect, if any, of iv paraquat on the lungs. Five control males were given tail vein injections of water only. The paraquat in water was formulated so that it could be administered at a constant volume of 0.0025 ml/g. The rats were anesthetized with ether and killed by cutting the aorta 13 days after treatment, and the lungs were examined grossly and microscopically.

Local instillations. In an effort to establish whether the effect of paraquat on the lung is a direct effect of the compound or the result of some metabolite formed elsewhere in the body, the material was instilled intrabronchially into male rats. The paraquat was dissolved in water in the appropriate concentrations so that the desired dosages could be injected at a constant volume of 0.0003 ml/g of body weight. Thirty drops of India ink per 100 ml of solution were added as a marker. Groups of 3 rats each were given 0.05, 0.1, 0.2, and 0.4 mg/kg of paraquat. Four control rats were given a similar solution without paraquat at 0.0003 ml/g. The dosing procedure for each rat was as follows: While the rat was under ether anesthesia, the trachea was exposed by making a midline incision in the ventral side of the neck, and a small puncture was made in the trachea with a 20-gauge hypodermic needle; a PE-10 polyethylene intramedic tube attached to the dosing syringe was then inserted and passed gently down the trachea; when resistance was felt the formulation was injected slowly. The rats were sacrificed by severing the aorta after ether anesthesia 7 days after dosing and the lungs were examined grossly and microscopically. Lung tissue from the area that contained carbon pigment in the control rats and the rats given 0.4 mg/kg of paraquat was studied for the presence of surfactant, using the procedure described by Mankelow (1967).

Since a local effect of paraquat did occur in the lungs, 0.134% paraquat solution marked with India ink was injected into the skeletal muscles of 10 male rats. With the rats under ether anesthesia, an incision was made in the skin of the upper thigh. The exposed muscle was injected with the solution at the rate of 0.006 ml/g of body weight to provide the rats with 0.8 mg/kg of paraquat. Ten control rats were treated in a similar manner with plain water marked with India ink. Two control rats and 2 treated rats each time were sacrificed after 2 and 4 weeks, and then at monthly intervals. The area of injection, identified by the presence of carbon, was removed and fixed for microscopic study.

RESULTS

Basic Toxicity Studies

The acute oral LD50 for paraquat in rats was 100 mg/kg (19/20 confidence limits, 87–117 mg/kg) in males and 110 mg/kg (90–134 mg/kg) in females. The acute dermal LD50 for paraquat was 80 mg/kg (60–96 mg/kg) in male rats and 90 mg/kg (74–110 mg/kg) in females.

The results of a 1-dose and 90-dose LD50 study for paraquat by the oral route in female rats are shown in Table 1. The chronicity factor (Hayes, 1967) of 5.2 for paraquat indicates that the compound has a moderate cumulative effect in this species. If a marked cumulative effect existed, a higher value would have been obtained, since only a very small portion of the 1-dose LD50 is necessary to kill half the test animals in 90 days. Paraquat has a moderate cumulative effect which is comparable to that of technical DDT (unpublished data).

TABLE I

ORAL TOXICITY OF PARAQUAT IN ADULT FEMALE RATS

Parameter	1-dose	90-dose
Number of rats tested	50	60
Survival time (days)	3–13	17–88
LD50[a]	110	21
19/20 Confidence limits[a]	90–134	19–23
Lowest dose to kill[a]	75	17
LD1[a]	45	13
Lowest dose causing clinical signs[a]	50	15
Lowest dose tested[a]	25	7.2[b]
Chronicity factor[c]	—	5.2

[a] Expressed as mg kg for 1-dose test or mg kg day for 90-dose test.
[b] Dietary concentration of 125 ppm.
[c] 1-dose LD50 ÷ 90-dose LD50.

Clinical signs of acute and chronic poisoning included diarrhea, wheezing, irregular and rapid breathing, and red stains around the snout. All animals that died showed morphological changes in their lungs. If the rats died within 5–6 days after a single oral dose, pulmonary edema, congestion, and intra-alveolar hemorrhage predominated. In animals that survived a single dose for 10 days or more, fibrosis predominated in the

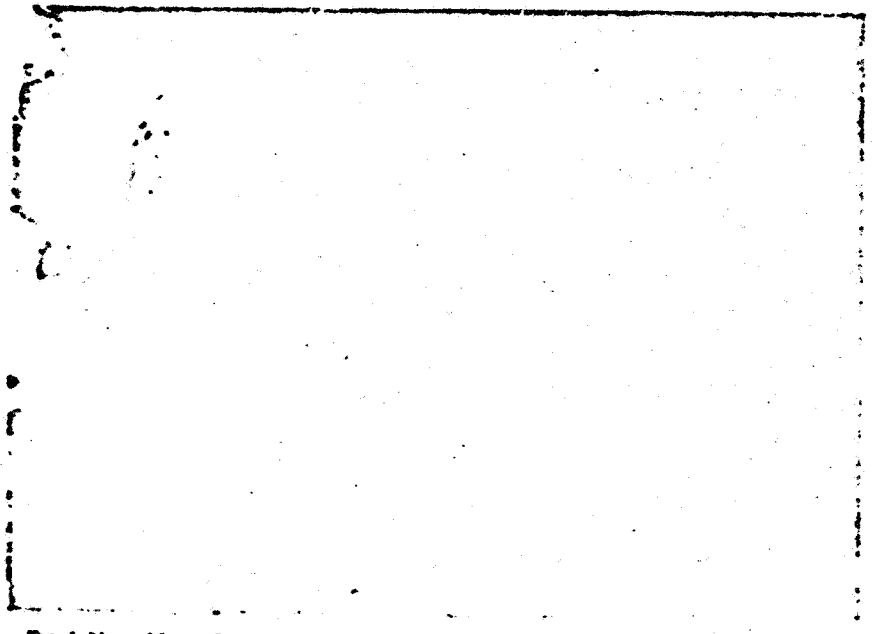

FIG. 1. Normal lung. C, [illegible]; B, [illegible]; E, [illegible]; M, [illegible]; G, [illegible]; A, [illegible]; A–C, [illegible]

lungs. Occasional multinucleated cells, epithelial proliferation, perivascular lymphocyte infiltration and squamous metaplasia were also observed.

All 33 animals that died in the 90-dose LD50 study had extensive diffuse fibrosis of the lung except for 1 rat which was given 300 ppm of paraquat. The lungs of this animal showed extensive intra-alveolar hemorrhage. Other findings in the lungs, but occurring less frequently than the diffuse fibrosis, were intra-alveolar hemorrhage, multinucleated epithelial cells, proliferation of the epithelium, periarteritis or perivascular lymphocyte infiltration, and a pink-staining homogeneous material lining the alveoli. The lungs of the rats that survived showed circumscribed areas of fibrosis which ranged in size from areas discernible only under the microscope to those measuring 1–2 cm in diameter.

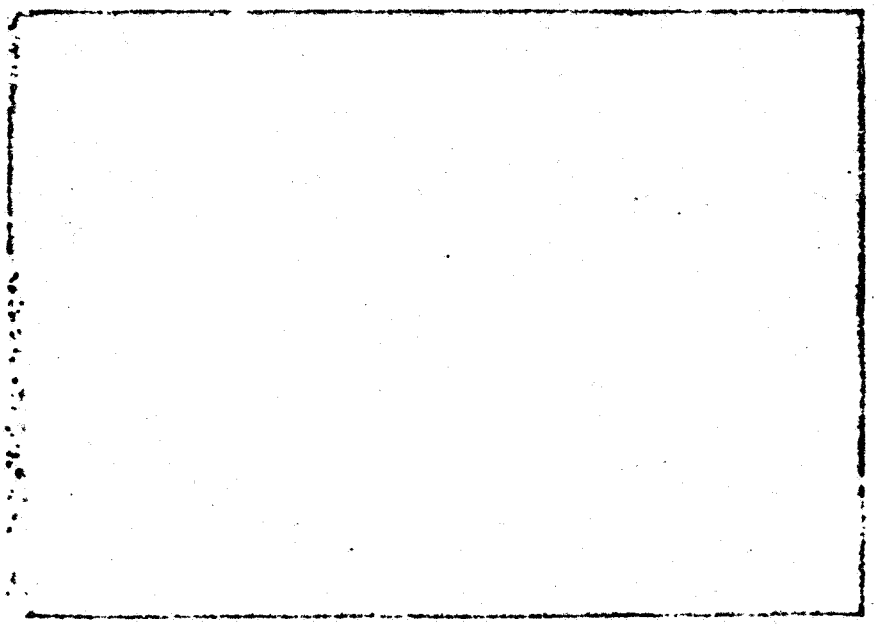

FIG. 2. Lung section from rat 4 hr after paraquat (400 mg/kg, po). Note pulmonary edema and red blood cell within alveolar space. *C*, capillary; *RBC*, red blood cell. Uranyl acetate stain, ×6000.

Lung tissue examined under the electron microscope 1 hr after paraquat administration by stomach tube at 400 mg/kg did not differ from that of the controls (Fig. 1). Four hours after a dose of 400 mg/kg, edema fluid and occasional red blood cells were observed within the alveolar spaces (Fig. 2). The granular pneumocytes were normal (Fig. 3). In some areas swelling of the cytoplasm of the membranous pneumocytes was observed (Fig. 4). Small dark granules normally present within the cytoplasm of membranous pneumocytes became more prominent.

Examination of lung tissue 43 hr after the animals were given paraquat by stomach tube at 120 mg/kg showed an increase in the prominent dark granules within the cytoplasm of the membranous pneumocytes that did not exhibit advanced stages of

degeneration. The alveolar lining cells contained large vacuoles within their cytoplasm (Fig. 5). Collagen and elastin were increased, the alveolar lining cells were separated by large spaces from the endothelial cells, and the cells exhibited degenerative change (Fig. 6).

In both control rats and rats given paraquat, a material of laminated or striated structure (Fig. 7) was observed within the alveoli; it probably represents pulmonary surfactant. In the animals killed 4 hr after paraquat administration, the appearance of this material did not differ from that of the control rats. Mendenhall *et al.* (1967) studied

FIG. 3. Lung section from rat 4 hr after paraquat (400 mg/kg, po). Note normal cytoplasm of granular pneumocyte. *LB*, lamellar body. Lead citrate stain. ×17,000.

the appearance of pulmonary surfactant under the electron microscope after centrifugation. Their material was not processed in the same manner as the lungs in our study, but their photomicrographs also suggest a lamellar structure of alveolar surfactant. This finding is also supported by the studies of Kikkawa *et al.* (1968) and Goldenberg *et al.* (1967).

The light microscopic examination of the lungs studied by the electron microscope 4 and 48 hr after paraquat administration showed intra-alveolar hemorrhage, perivascular lymphocyte infiltration, and prominent alveolar epithelial cells with dark nuclei.

Intravenous injection of paraquat at 6.0 mg/kg in male rats killed 2 of 5 rats tested

and produced extensive fibrosis in the lungs of the survivors. The 5 rats given 3.0 mg/kg iv survived; their lungs were normal grossly and microscopically.

Local Instillations

Intrabronchial injections of solutions of paraquat marked with India ink were limited to 1 or 2 lobes of the lung, but not always to the same lobes. The circumscribed areas were easily discernible at autopsy 7 days after injection. The lung tissue in this area

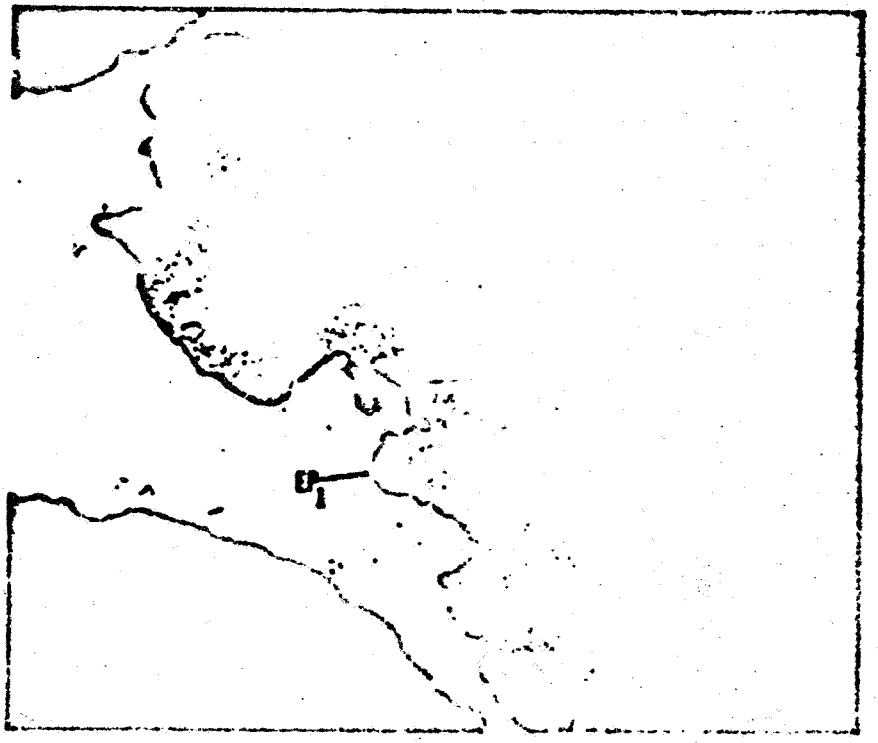

FIG. 4. Lung section from rat 4 hr after paraquat (400 mg/kg, po). Note swelling of the cytoplasm of the membranous pneumocyte. *EP*, membranous pneumocyte; *BM*, basement membrane; *E*, elastin; *EN*, endothelial cell; *RBC*, red blood cell. Lead citrate and uranyl acetate stain. ×25,000.

showed carbon deposits microscopically. The lowest dosage level of paraquat given, 0.05 mg/kg, produced fibrosis and epithelial proliferation in the areas that contained the carbon pigment (Fig. 8). This amount was 120 times less than the dose necessary to produce lung lesions when the material was injected into the tail vein. The remaining lung tissue not containing carbon pigment was normal. Carbon pigment but no other morphologic changes were observed in lung tissue of controls which had been given carbon pigment in water intrabronchially. Frozen sections of lung tissue from rats that

had been injected with 0.4 mg/kg of paraquat and carbon did not show air bubbles on microscopic examination. Air bubbles were observed in the surrounding lung tissue and also in lung tissue that had only been injected with carbon pigment in water.

A single im injection of paraquat marked with carbon resulted in circumscribed areas of fibrosis in the muscle at the site of the carbon deposit. These areas decreased in size with time and consisted of thin scarlike strands of fibrous tissue 4 and 5 months after injection. Carbon pigment suspended in water was injected into control animals and remained localized; fibrosis was not observed in these areas. The dose used for the im injections was only 7.5 times less than the dose necessary to produce lung lesions by iv injection of paraquat.

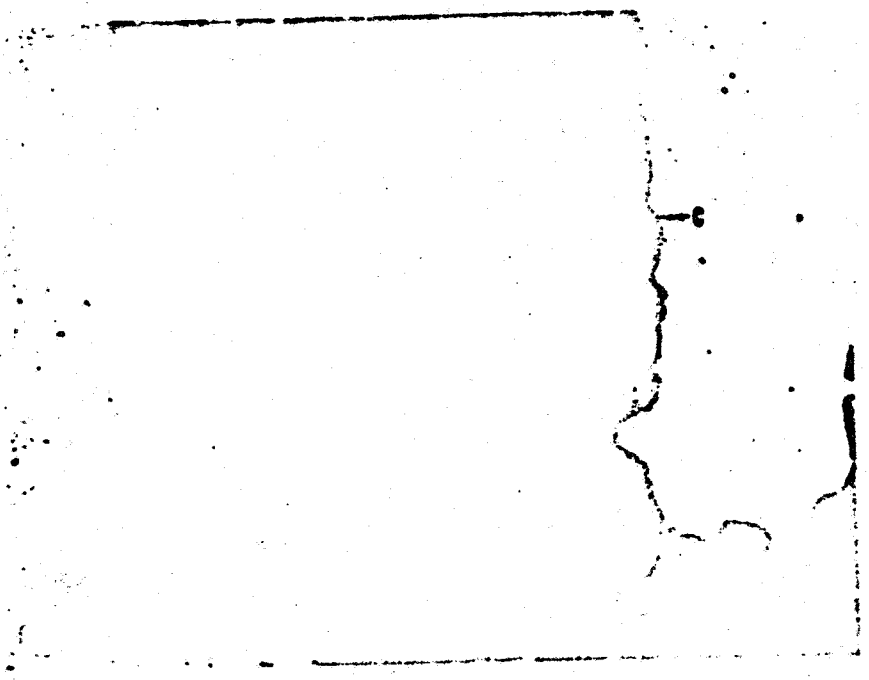

FIG. 5. Lung section from rat 48 hr after paraquat (150 mg/kg, po). Note the large vacuoles within the cytoplasm of the membranous pneumocytes and the prominent dark granules. *Ep*, membranous pneumocyte; *V*, vacuoles; *R*, dark prominent granules; *C*, collagen. Lead citrate and uranyl acetate stain. ×22,600.

DISCUSSION

An effect of paraquat on lung tissue is again demonstrated. If a high single dose is given, intra-alveolar hemorrhage occurs and the rat dies in 5–6 days. At a lower dose the rats die later and fibrosis of the lung predominates. Repeated oral doses of this compound cause an effect which, after a period of time, lead to fibrosis of the lung. The [illegible] factor, obtained by dividing the 1-dose LD50 by the 90-dose LD50 (Hayes, 1967), is

5.2, a value which is comparable to that of technical DDT (unpublished data). Local single instillations either into the lung or the skeletal muscle also result in fibrosis. The lowest iv dose causing lung fibrosis was 6 mg/kg but a dose 120 times less than that instilled locally into the lung produced fibrosis. This observation shows that the lungs are highly sensitive to paraquat and that the compound has a direct effect on the lungs. Since the lung lesions can be caused by injecting a small amount of paraquat directly into the lungs, they are a result of the compound itself and not of a metabolite formed elsewhere in the body. The fibrosing effect of paraquat is also demonstrated by its effect after injection into skeletal muscle, but here a much higher dose was employed. The areas in the lung showing the fibrosis lose their surfactant (Manktelow, 1967). The effect on the

FIG. 6. Lung section from rat 48 hr after paraquat (150 mg kg, po). Note wide spaces between epithelial and endothelial cells and the swelling of the cytoplasm. *C*, capillary; *A*, alveolus; *RBC*, red blood cell; *COL*, collagen; *EN*, endothelial cell; *EP*, membranous pneumocyte. Lead citrate and uranyl acetate stain. ×8370.

surfactant is not necessarily the primary change produced in the lungs by paraquat, since any area of lung tissue with hemorrhage or fibrosis severe enough to alter the alveolar structure would very likely cause the surfactant to disappear.

Electron microscopic examination showed an increase in collagen within the intercellular spaces, the formation of elastin, and widening of the intercellular spaces. Granules resembling ribosomes become larger and more prominent in the cytoplasm of membranous pneumocytes. In some areas of the lung the alveolar lining cells show advanced stages of swelling and degeneration 2 days after a single high dose of paraquat.

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The findings in this study do not explain why the lung is the primary target organ for paraquat. Kapany *et al.* (1969) found that breathing pure oxygen at 1 atmosphere of pressure almost completely destroyed alveolar lining cells in monkeys. The destroyed membranous pneumocytes (type 1) were replaced by granular pneumocytes (type 2). Thickening of the air-blood tissue space was caused by an increase in granular pneumocytes and interstitial fibers. The proliferation of the granular pneumocytes was not observed in our study at the dosage levels and time intervals investigated.

Klaus *et al.* (1962) found evidence which supports the hypothesis that the surfactant

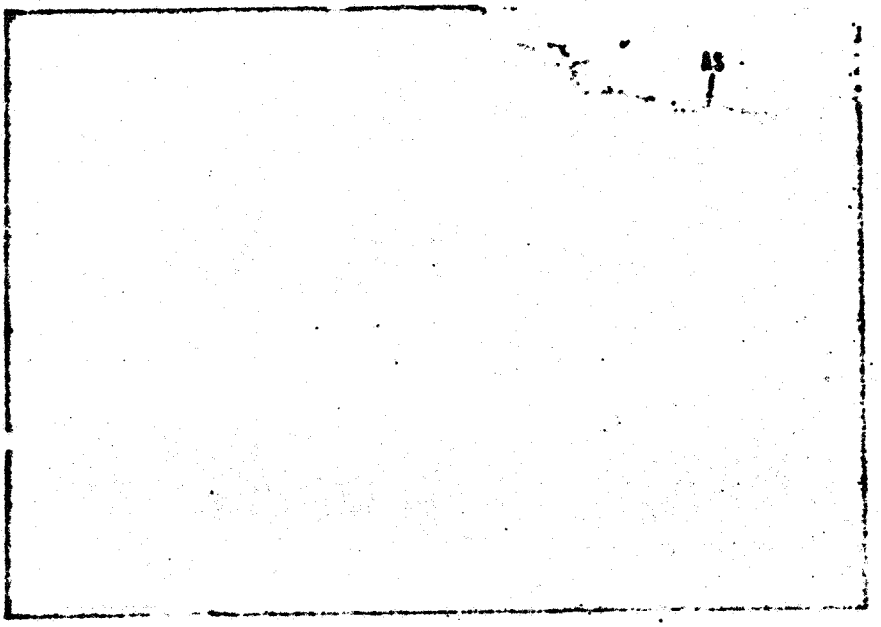

FIG. 7. Lung section illustrating striated structure of alveolar surfactant (*AS*). This material is observed in both normal and paraquat-treated animals. Lead citrate stain. ×15,400.

of the lungs develops during the process of transformation of mitochondria to lamellar bodies in the granular pneumocytes. Macklin (1954) also postulated that the pulmonary surfactant is secreted by the granular pneumocytes. According to Schaefer *et al.* (1963), the lamellar bodies in the granular pneumocytes are responsible for the secretion of alveolar surfactant. These bodies were not affected 4 hr after the administration of paraquat. Likewise, the mitochondria of the granular pneumocytes appeared to be normal 4 hr after an oral dose of paraquat, while ultrastructural changes were observed in the membranous pneumocytes. The lamellated material within the alveoli (Fig. 7), which most likely represents surfactant, was also unaltered. Very early ultrastructural changes of the cytotoxic principle representing or related to pulmonary edema could therefore not be demonstrated.

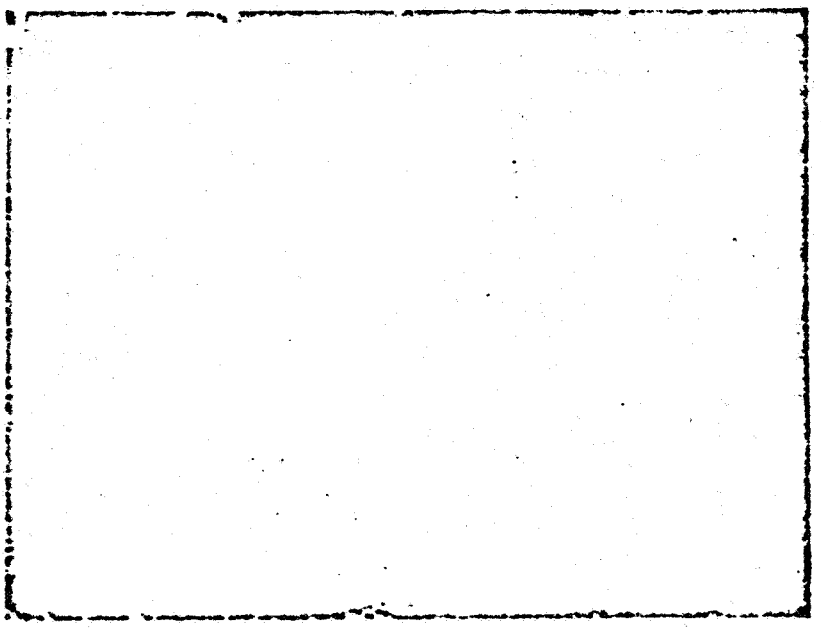

FIG. 8. Lung section illustrating fibrosis and epithelial proliferation. Hematoxylin and eosin stain. ×125.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Ralph E. Linder, Mr. Richard L. Moore, Mrs. Estelle Gray, Mrs. Annie Alford, and Mrs. Mary Anne Dobbs for their valuable assistance in these experiments, and Miss Cary Calloway of the National Communicable Disease Center for technical assistance with the electron microscopic studies.

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