

## Effect of Paraquat-Induced Lung Damage on Permeability of Rat Lung to Drugs<sup>1</sup> (39194)

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Paraquat, a widely used herbicide, has been shown to have a specific cytotoxic effect on mammalian lungs (1). Pulmonary lesions resulting from oral or parenteral administration of the compound include atelectasis, hyaline membrane formation, hemorrhage, interstitial inflammation, and edema (2-5). Some investigators have suggested that the damage is associated with lung surfactant deficiency (4, 6, 7). Because of the various pathologic changes seen in the paraquat-damaged lung, lesions produced by this agent have been postulated to resemble respiratory distress syndrome of the newborn (4, 6), interstitial pulmonary fibrosis (8), and pneumonia (3).

Although considerable research has dealt with the morphologic and biochemical changes that occur in paraquat-damaged lungs (5, 9-11), little work has been done to investigate the effect of these changes on lung permeability. The present study in the rat describes quantitatively the pulmonary absorption of three drugs from the paraquat-damaged lung. The drugs include an acid, a base, and a quaternary ammonium compound. Comparison of altered drug absorption rates with the time course of lung damage provides information concerning changes in membrane permeability.

**Materials and methods.** Lung damage was produced in male Charles River-derived rats (125-275 g) by oral administration of paraquat dichloride (250 mg/kg) in 0.9%

NaCl solution. Control animals received an equivalent volume of drug-free saline solution. At various times up to 30 days after treatment, lung damage was assessed and pulmonary drug absorption rates were measured in both paraquat-treated and control animals.

For histologic assessment of lung damage, sections of lung tissue from paraquat-treated and control animals were examined by light microscopy. To obtain the tissues, animals were anesthetized with pentobarbital (50 mg/kg), the trachea was cannulated with PE 240 tubing, and the lungs were inflated under a pressure of 12-13 cm H<sub>2</sub>O with 10% formalin in phosphate buffer (pH 7.4). After fixation, tissue sections (6  $\mu$ m) were cut from paraffin blocks and stained with hematoxylin and eosin.

A quantitative assessment of the degree of lung damage was obtained from the wet and dry tissue weights of paraquat-treated and control animals. After removal of the lungs and trachea from an anesthetized animal, the tissue was blotted on moistened filter paper, minced into tared weighing vials, weighed, and then dried at 100° to constant weight.

To investigate the effect of paraquat-induced lung damage on lung permeability, drug absorption rates were measured at various times after paraquat or saline (control) administration according to a method described previously (12). Briefly, animals were anesthetized with pentobarbital, and the trachea was exposed through a ventral midline incision in the neck. A 2.5-cm length of PE 240 tubing, which served as a tight-fitting tracheal cannula, was inserted to a depth of 0.6 cm through an incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage. The cannula was left in place during the entire experiment. Solutions (10 mM) of procaine amide

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ethobromide HBr (PAEB), *p*-aminohippuric acid (PAH), and procaine amide HCl were prepared in Krebs-Ringer phosphate solution (pH 7.4), in which the Ca ion had been lowered to one-fifth the usual concentration to avoid turbidity (13). One-tenth milliliter of drug solution was injected into the lungs through PE 20 tubing attached to a calibrated 100- $\mu$ l syringe. The injection tubing was inserted through the tracheal cannula to a point approximately 1 mm above the bifurcation of the trachea (12), the drug solution injected over a 1-2-sec interval, and the tubing quickly withdrawn. The incision in the skin was then closed with a wound clip after drawing the skin up close to the sides of the tracheal cannula. Body temperature was maintained at  $37 \pm 1^\circ$  by heat from a 40-W incandescent lamp suspended above the animal.

At the end of an absorption period (3-60 min), the lungs and attached trachea were excised from the animal, weighed, and placed in a 15-ml Tenbroeck glass homogenizer together with sufficient distilled water to make a total fluid volume (tissue water plus distilled water) of 11 ml (12). After thorough homogenization, 1 ml of concentrated HCl and 1.2 ml of 50% trichloroacetic acid solution were added to precipitate proteins, and the sample was again homogenized. The homogenate was then centrifuged for 20 min at 600g. Aliquots of the resulting clear supernatant fluid were assayed for drug according to the colorimetric procedure of Bratton and Marshall (14). When known amounts of PAEB, PAH, or procaine amide were added to lung tissue and the assay carried out as described above, drug recoveries were 93, 95, and 92% (SE  $\pm$  1 in six determinations), respectively. Results were accordingly corrected for the incomplete recoveries.

Paraquat dichloride was obtained from Imperial Chemical Industries and *p*-aminohippuric acid from Eastman Organic Chemicals. Procaine amide HCl and procaine amide ethobromide HBr were kindly provided by the Squibb Institute for Medical Research.

**Results and discussion.** Lung damage produced by paraquat in the present study appeared to be similar to that reported previ-

ously by other investigators (1, 2, 4, 5, 9). Shown in Fig. 1 are sections of lung tissue taken from rats at 1, 3, 5, and 8 days after paraquat administration. Lungs obtained from rats at 0.5-2 days after paraquat treatment appeared normal. However, by the third day, evidence of hemorrhage began to appear, and perivascular edema together with the first signs of tissue inflammation could be seen histologically. After 5-8 days, lungs from paraquat-treated rats were greatly enlarged, and nearly the entire lung surface had taken on a dark brownish-red color indicative of severe hemorrhage and congestion. Edema and inflammation worsened leaving little functional tissue for pulmonary gas exchange. By the 15th day, however, lungs from surviving paraquat-treated rats appeared normal again except for a slight hyperemia.

Increased lung weight (Fig. 2) and water content (Fig. 3) in paraquat-treated animals paralleled the histologic changes described above. For example, the wet weight increased nearly fourfold by the fifth day after paraquat administration; and the dry weight, which increased as much as threefold, was maximal on the eighth day. Similarly, the water content of lung tissue increased from 78 to 87% of the wet tissue weight by the fifth day after paraquat administration. By the 15th day, lung water content as well as the wet and dry tissue weights had returned to near control values.

The permeability of the lung to drugs was markedly increased in the presence of paraquat-induced damage. For example, the pulmonary absorption rates for the three compounds studied were increased by 1.4-2.8-fold in paraquat-treated animals as compared to controls (Figs. 4-6). As shown in Fig. 4, the 60-min absorption of the quaternary amine PAEB was 35-45% of the administered dose in control animals, but the value rose to 90-96% of the dose on the third to fifth day after herbicide treatment. Similarly, as shown in Fig. 5, the 45-min absorption of the organic anion PAH amounted to 40-50% of the dose in controls but rose to 92-97% of the dose on the third to eighth day after paraquat treatment. Although the lipid-soluble tertiary amine procaine amide also showed an increased ab-

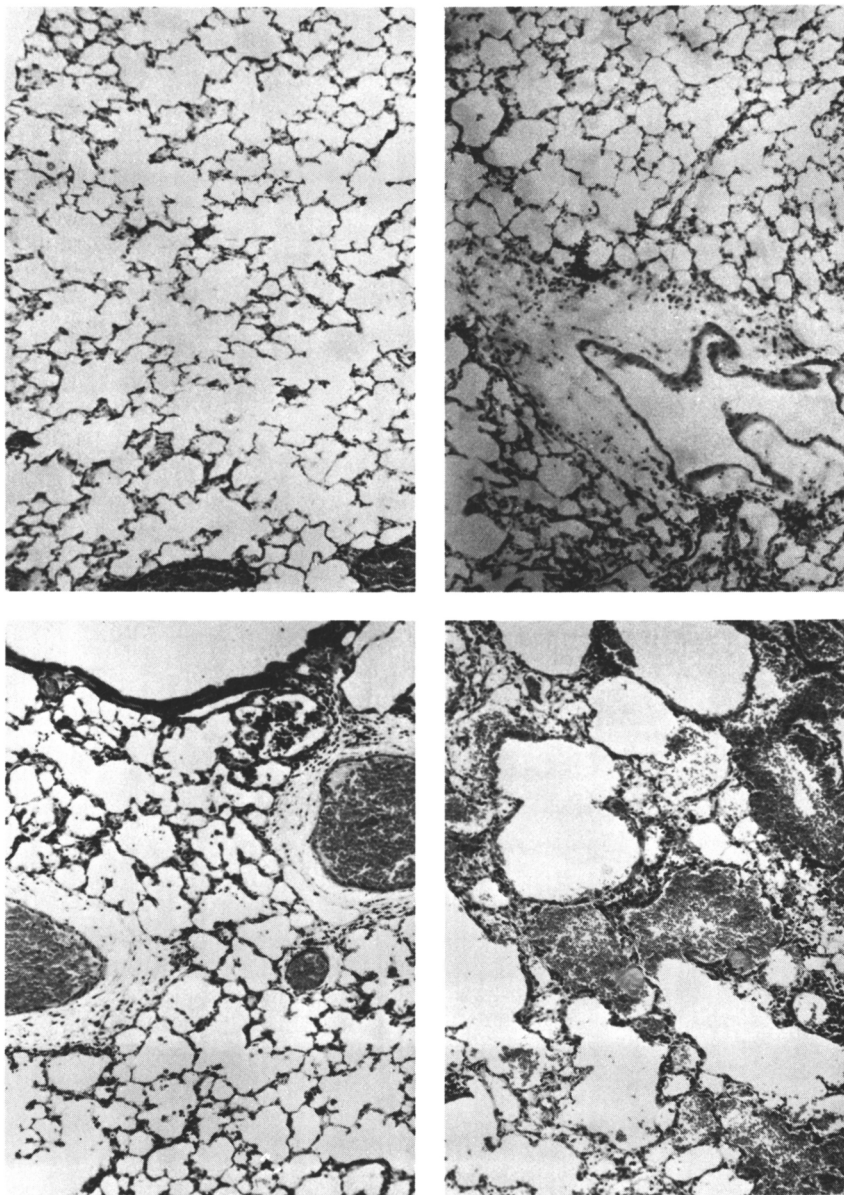


FIG. 1. Lung sections ( $6\ \mu\text{m}$  thick) stained with hematoxylin and eosin from rats given an oral dose ( $250\ \text{mg}/\text{kg}$ ) of paraquat ( $100\times$  magnification). Tissue sample taken at 1 day after paraquat treatment (upper left) appears normal as compared with tissue taken at 3 days after treatment (upper right), which shows evidence of hemorrhage and perivascular edema together with the first signs of inflammation. At 5 days after paraquat administration (lower left), edema, hemorrhage, and inflammation are increased, and at 8 days (lower right) little functional tissue remains for gas exchange.

sorption rate in paraquat-damaged lungs (Fig. 6), the difference from controls was not as great as with the other two drugs. For example, the 3-min absorption of procaine amide 3–5 days after animal treatment was

47–55% in controls and 72–78% in paraquat-treated rats.

An increase in membrane porosity in paraquat-damaged lungs could well account for the increased rates of drug absorption seen

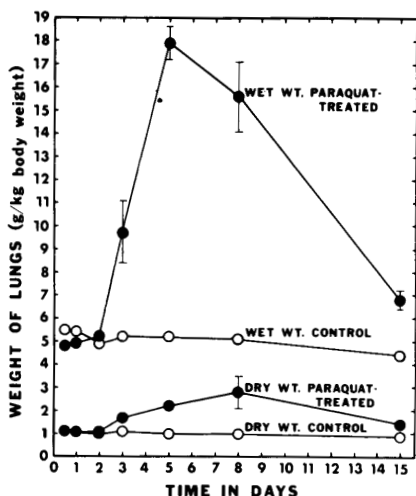


FIG. 2. Wet weight and dry weight of lungs from rats 0.5-15 days after oral administration of paraquat (250 mg/kg) or an equivalent volume of saline (control). Each point represents the mean value for four to six animals. Brackets indicate SE wherever large enough to be shown.

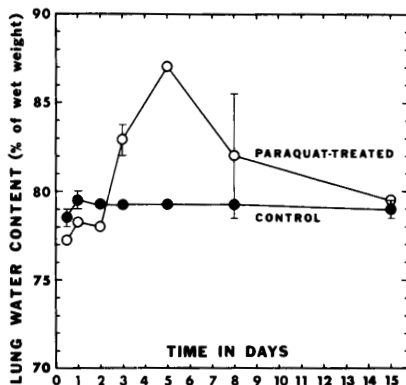


FIG. 3. Water content of lungs from rats 0.5-15 days after oral administration of paraquat (250 mg/kg) or an equivalent volume of saline (control). Each point represents the mean value for four to six animals. Brackets indicate SE wherever large enough to be shown.

in the present study. For example, previous work in this laboratory with the normal rat lung (12, 13) indicated that relatively lipid-insoluble compounds, such as PAEB and PAH, are absorbed primarily by diffusion through aqueous membrane pores. The markedly increased absorption rates seen for these compounds in paraquat-damaged lungs is thus consistent with the idea of an

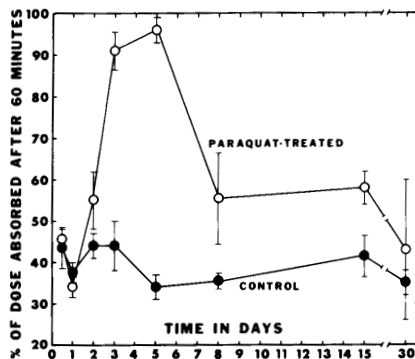


FIG. 4. Pulmonary absorption (60 min) of 10 mM PAEB in rats 0.5-30 days after oral administration of paraquat (250 mg/kg) or an equivalent volume of saline (control). Each point represents the mean value  $\pm$  SE for three animals.

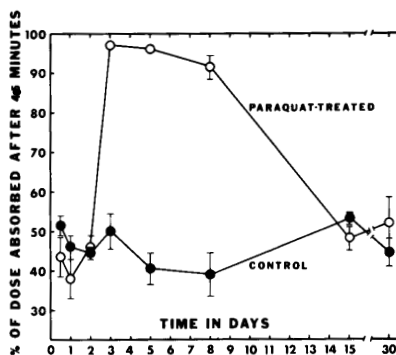


FIG. 5. Pulmonary absorption (45 min) of 10 mM PAH in rats 0.5-30 days after oral administration of paraquat (250 mg/kg) or an equivalent volume of saline (control). Each point represents the mean value for three animals. Brackets indicate SE wherever large enough to be shown.

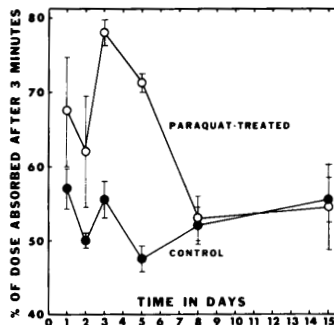


FIG. 6. Pulmonary absorption (3 min) of 10 mM procaine amide in rats 1-15 days after administration of paraquat (250 mg/kg) or an equivalent volume of saline (control). Each point represents the mean value  $\pm$  SE for three to five animals.

increased porosity for the absorbing membrane. In the case of procaine amide, a lipid-soluble drug, increased membrane porosity would be expected to have a lesser effect on the absorption rate, since, although diffusing through pores, the drug is absorbed predominantly by crossing lipid regions of the pulmonary membrane (13).

Although increased membrane porosity may be a significant factor in the increased permeability of paraquat-damaged lungs to drugs, other factors could contribute to changes in membrane permeability. For example, pulmonary surfactant activity has been shown to be reduced in paraquat-damaged lungs (6, 7), and this might alter lung membrane permeability since the application of synthetic surface-active agents to certain biologic membranes has been shown to alter rates of drug penetration (15). While a change in pulmonary blood flow that might result from paraquat treatment could also be an important factor in pulmonary absorption, it would not account for the increased drug absorption rates seen in the present study, since absorption of these compounds is not blood-flow limited (13).

The degree to which drug absorption is increased after paraquat-induced lung damage may give an underestimate of the actual increase in lung membrane permeability, since the presence of edema fluid would be expected to slow absorption rates owing to the decreased absorbing surface/volume ratio.

**Summary.** To investigate the effect of paraquat-induced lung damage on pulmonary absorption of drugs, rats were given a single oral dose of paraquat (250 mg/kg), and rates of drug absorption from damaged and control lungs were compared after various times. To measure absorption rates of drugs, 0.1 ml of 10 mM drug solution was administered through a tracheal cannula to anesthetized animals and, after various times, lungs were assayed for unabsorbed compound. Drugs investigated were pro-

caine amide ethobromide, *p*-aminohippuric acid, and procaine amide. Rates of drug absorption increased 1.4–2.8-fold at 3–5 days after paraquat administration and returned to near control values by the 15th day. The results suggest that paraquat-induced lung damage increases the porosity of the pulmonary epithelium.

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