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EFFECT OF PARAQUAT TREATMENT OF RATS ON DISPOSITION OF 5-HYDROXYTRYPTAMINE AND ANGIOTENSIN I BY PERFUSED LUNG

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Abstract—Rats were injected with the herbicide, paraquat dichloride (25 mg/kg, i.p.), and their lungs were perfused 2–28 days later. Isolated lungs from rats treated with paraquat (PQ) 3 or 4 days before perfusion removed significantly less perfused 5-hydroxytryptamine (5-HT) than did saline-injected controls. This effect was not caused by PQ directly, since perfusion of lungs from untreated animals with PQ did not alter removal of co-perfused 5-HT. Monoamine oxidase activity of 600 *g* supernatant fractions of homogenates of lungs from PQ-treated rats was also reduced compared to controls. Although removal of perfused angiotensin I (1 ng/ml) by isolated lung was not altered by PQ pretreatment, angiotensin-converting enzyme activity in 600 *g* supernatant fractions of lung homogenates was reduced significantly. These results suggest that PQ damages pulmonary endothelium and impairs the metabolic function of lung.

Pulmonary lesions in man and in experimental animals result from exposure to the herbicide, paraquat (1,1′-dimethyl-4,4′-bipyridinium chloride, methyl viologen). Since the pulmonary sequelae following paraquat ingestion in man are similar to those in the rat [1, 2], this species has been used extensively in studies of the pathology of paraquat (PQ) poisoning. In the rat, morphologic changes in alveolar epithelium, including damage to both type I and type II alveolar pneumocytes, have been commonly observed after PQ administration. Disagreement exists, however, as to whether PQ also alters vascular endothelium of lung. Based on electron microscopic studies in rats, some investigators [3–5] have reported paraquat-induced lesions of varying severity in alveolar capillary endothelium, including swelling of endothelial cytoplasm, vacuolization, lipid inclusions and swollen mitochondria. Others [6, 7], however, have reported that alterations in endothelium do not occur in paraquat poisoning.

Lung is known to remove from the circulation and to metabolize a wide variety of endogenous vasoactive agents as well as many drugs [8–12]. For example, circulating angiotensin I is rapidly converted by lung to angiotensin II [13]. Studies by Ryan *et al.* [14] have indicated that pulmonary angiotensin-converting enzyme activity is associated with the luminal membrane of alveolar capillary endothelial cells. In addition, certain biogenic amines are rapidly removed from the circulation by lung and metabolized [15]. For example, 70–90 per cent of circulating 5-hydroxytryptamine (5-HT) is removed in a single pass through perfused lungs from a variety of species [16–20], including the rat [21, 22]. 5-HT is taken into lung by a facilitated transport process and metabolized by monoamine oxidase [17, 21–23]. Autoradiographic and histofluorescence studies [16, 24, 25] have localized the site of uptake of 5-HT to the pulmonary endothelium.

If morphologically demonstrable injury to alveolar endothelium occurs in PQ poisoning, as some investigators have reported, one might expect the metabolic function of these cells to be compromised. Indeed, recent studies [26, 27] employing toxicants which indisputedly produce damage to pulmonary capillary endothelium suggest that impaired removal of 5-HT by perfused lung may be an early and sensitive index of endothelial cell damage. Accordingly, we have examined the ability of isolated lungs from paraquat-treated rats to clear perfused 5-HT or to metabolize angiotensin I.

MATERIALS AND METHODS

Treatment of animals. Male Sprague–Dawley rats (Spartan Farms, Haslett, MI, U.S.A.) weighing 170–240 g were housed on wood chips in plastic cages and allowed food and water *ad lib.* for the duration of the study. Rats were injected i.p. either with 25 mg/kg of paraquat dichloride or with an equal volume of saline. The PQ injection solution was prepared fresh daily by dissolving paraquat dichloride in saline and adjusting the pH to between 7.0 and 8.0. All treated animals were observed for periods ranging between 2 and 28 days, following which time they were killed and their lungs were used either in perfusion studies or for enzyme assays.

Perfusion of rat lungs. Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and given 500 U of heparin, i.v., to prevent clotting. The pulmonary artery and the trachea were cannulated *in situ*, and the lungs were carefully removed from the animal and placed in the perfusion apparatus, which was maintained at 37°. During surgery and immediately after placement in the perfusion apparatus, the lungs were inflated and deflated several times to prevent atelectasis. During subsequent perfusion, the lungs were statically inflated with 2 ml of room air. The lungs were perfused, using a single-pass (non-recirculating) system, at a rate of 10 ml/min with

Krebs bicarbonate perfusion medium [28] at 37° containing 4% bovine serum albumin and aerated with 95% O_2/5% CO_2. Perfusion pressure was monitored continuously by means of a Grass model 7 polygraph with a P23ID Statham pressure transducer.

In studies involving rats pretreated with PQ, lungs were perfused for a short stabilization period with perfusion medium alone, after which they were perfused with medium containing 0.1 μM [^{14}C]-5-hydroxytryptamine for 4 min. During the final 30 sec of this perfusion period, a sample of the effluent was collected for analysis of amines and metabolites. The perfusion medium was then changed to one containing 1 ng/ml of angiotensin I. The lungs were perfused for an additional 4 min with this perfusion medium; a sample of the effluent perfusion medium was collected during the final 30 sec of perfusion for analysis of angiotensin I. The lungs were then removed from the perfusion apparatus, gently blotted and weighed.

Analysis of [^{14}C]-*5-hydroxytryptamine* ([^{14}C]-*5-HT*). The methods used for analysis of radiolabeled amines and their metabolites have been described in detail previously [29, 30]. To separate [^{14}C]-5-HT and [^{14}C]-5-hydroxyindolacetic acid (5-HIAA), its deaminated metabolite, 0.5-ml aliquots of lung effluent were passed through columns of Bio-Rex 70 cation exchange resin (sodium form, pH 6.0) after with the [^{14}C]-5-HIAA was eluted from the columns with 2.5 ml water. Radioactivity in the resultant samples was determined by liquid scintillation spectrometry. Removal of 5-HT was calculated as the difference between the radioactivity of the unchanged amine in the inflow perfusion medium and that in the collected effluent. Per cent removal (% R) of perfused 5-HT was calculated as:

$$\% R = \frac{C_{a,i} - C_{a,o}}{C_{a,i}} \times 100$$

where $C_{a,i}$ and $C_{a,o}$ represent concentrations of ^{14}C-amine in the inflow and effluent perfusion medium respectively. Similarly, the per cent metabolite (% M) appearing in the effluent was calculated as:

$$\% M = \frac{C_{m,o}}{C_{a,i}} \times 100$$

where $C_{m,o}$ represents the concentration of metabolite in the effluent.

Analysis of angiotensin I. The concentration of angiotensin I (AI) was determined by radioimmunoassay employing an antibody to pure [Asp^1, Ile^5]-AI [31]. After incubation of the sample at 4° for 18 hr with antibody and Asp^1, [^{125}I]-Ile^5-angiotensin I, free and antibody-bound AI were separated by activated charcoal (10% suspension) binding of free AI followed by centrifugation. Resultant fractions were counted in a gamma scintillation counter and the results were used to calculate the concentration of AI in the original sample.

Determination of monoamine oxidase (MAO) activity and angiotensin-converting enzyme activity. Rats were decapitated, and their lungs were removed, weighed and homogenized at 4° in 4 vol. of a buffer containing 300 mM NaCl and 100 mM potassium phosphate (pH 7.0) using a polytron homogenizer (30 sec, speed setting 5). The homogenate was centrifuged at 600 *g* for 10 min at 4°, and the resultant supernatant fraction was analyzed for monoamine oxidase and angiotensin-converting enzyme activity.

[^{14}C]-5-HT was used as substrate for the determination of pulmonary monoamine oxidase activity. The reaction mixture consisted of 0.2 μmole of [^{14}C]-5-HT in a total of 2 ml of 0.05 M potassium phosphate buffer (pH 7.0) containing 150 mM NaCl. An aliquot (0.2 or 0.3 ml) of the 600 *g* supernatant fraction of lung was added and the mixture was incubated at 37° for 15 min. Deamination of 5-HT under these conditions was linear during the 15-min incubation period. The reaction was stopped by the addition of 0.2 ml of 0.2 M $Ba(OH)_2$ followed by 0.2 ml of 0.2 M $ZnSO_4$. The mixture was then centrifuged at 3000 *g* for 10 min, and the resulting supernatant fraction was analyzed by cation exchange chromatography for [^{14}C]-5-HT and its acid metabolite, [^{14}C]-5-HIAA, as described above.

Angiotensin-converting enzyme (ACE) activity was determined by a modification of the method described by Cushman and Cheung [32]. Enzyme incubation was initiated by the addition of 0.10 ml of the 600 *g* supernatant fluid of lung homogenates to a 0.15 ml hippuryl-L-histidyl-L-leucine (HHL) solution. HHL was prepared in 100 mM potassium phosphate–300 mM sodium chloride buffer (pH 8.3) and was added to the reaction mixture to give a final concentration of 5.0 mM. Following a 30-min incubation at 37°, 0.25 ml of 1 N HCl was added to stop the reaction. The hippuric acid formed was extracted into 1.5 ml ethyl acetate, then centrifuged at 2000 *g* for 10 min. A 1.0-ml aliquot of the acetate phase was then evaporated at 40° under nitrogen gas. The residual hippuric acid was dissolved in 3.0 ml of 1 M NaCl and the optical density was determined at 228 nm. A molar extinction coefficient of 9.8 mM^{-1} cm^{-1} was used to convert to molar units. Protein was measured by the method of Lowry *et al.* [33].

Statistics. Results were expressed as $\bar{X} \pm$ S.E.M. and were analyzed statistically by Student's *t*-test [34]. The level of significance was taken to be $P < 0.05$.

Drugs, reagents and isotopes. 5-Hydroxy-[2-^{14}C]-tryptamine creatinine sulfate (58 mCi/m-mole) was purchased from the Amersham/Searle Corp., Arlington Heights, IL; paraquat dichloride and 5-hydroxytryptamine creatinine sulfate complex from the Sigma Chemical Co., St. Louis, MO; Bio-Rex 70 cation exchange resin (100–200 mesh) from BioRad Laboratories, Richmond, CA; bovine albumin powder (fraction V) from Miles Laboratories, Inc., Elkhart, IN; HHL from Vega Fox Biochemicals, Tucson, AZ; Asp^1, [^{125}I]-Ile^5-angiotensin I from the New England Nuclear Corp., Boston, MA; and angiotensin I standards from E. R. Squibb & Sons, Inc., Princeton, NJ.

RESULTS

The time course of lethality to rats following a single intraperitoneal injection of 25 mg/kg of paraquat dichloride is presented in Fig. 1. Most deaths occurred between 1 and 3 days after PQ treatment.

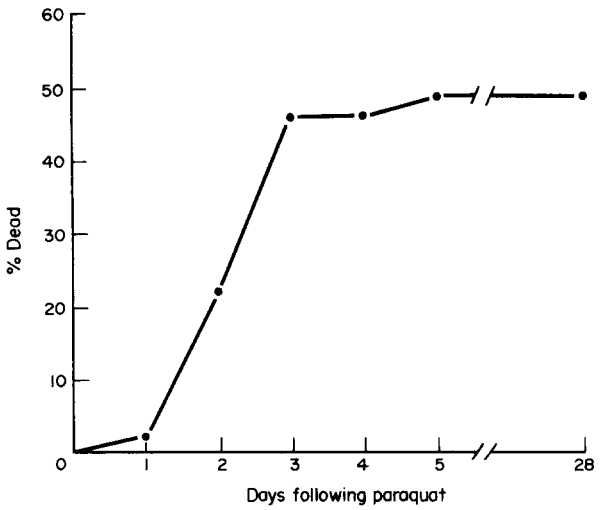

Fig. 1. Lethality following treatment of rats with 25 mg/kg of paraquat dichloride. Forty-one rats were given one intraperitoneal injection of paraquat dichloride on day zero and observed for 10–28 days.

Between days 5 and 28 no deaths occurred. The changes in body weight occurring in animals which survived following PQ treatment are presented in Fig. 2. Animals lost weight during the first 3 days after PQ treatment but subsequently recovered the initial weight loss so that by day 28 weights of the PQ-treated and saline-treated animals were the same.

The lung weights of the animals killed at various times after PQ treatment for use in lung perfusion studies are presented in Table 1. A statistically significant increase in lung wet weight was observed only at 3 days after PQ treatment. In isolated lung preparations from these animals, no large differences were observed in perfusion pressure between saline-treated and PQ-treated groups. In the lung preparation which we employed, accumulation of fluid by lung is invariably associated with an increase in inflow pressure during the course of perfusion. Such changes in perfusion pressure of lungs from PQ-treated rats were small and not different from those of saline controls (Table 1).

To determine if PQ treatment alters pulmonary disposition of 5-HT, perfused lungs from rats treated 2–28 days previously with PQ were examined for their ability to remove perfused [^{14}C]-5-HT. A significant decrease in the fraction of 5-HT removed in a single pass was observed in lungs from rats treated with PQ 3 or 4 days prior to perfusion (Table 2). The decrease in 5-HT clearance occurring on day 3 was accompanied by a decrease in the amount of metabolite appearing in the effluent. These results prompted an examination of monoamine oxidase activity in homogenates of lungs from PQ-treated animals. Table 3 shows that 3 days after PQ treatment monoamine oxidase activity was depressed whether results were expressed as specific activity (activity/mg of protein), normalized to lung weight or expressed on a whole organ basis. A small decrease in MAO activity was noted on day 4 when the data were expressed on a whole lung basis. This was not due to altered recovery of MAO in the 600 g supernatant fraction since the ratio of activity in the supernatant

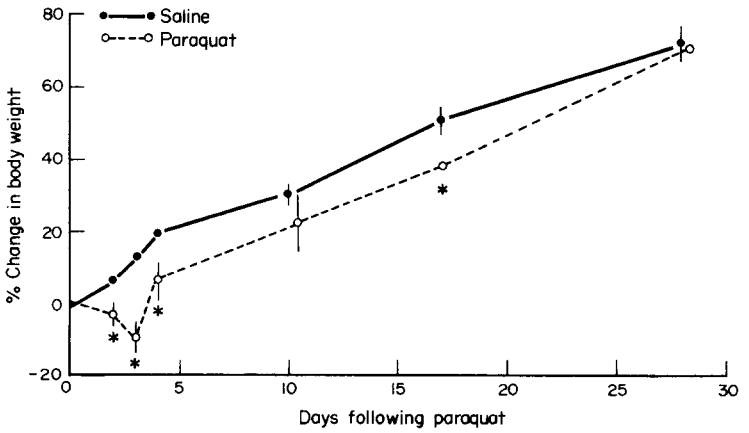

Fig. 2. Effect of paraquat treatment on body weight. Rats initially weighing 209 ± 2 g were injected i.p. once with paraquat (25 mg/kg, open circles) or with saline (closed circles) on day zero. Each symbol represents mean ± S.E. of one group of four to thirteen animals. The asterisk (*) indicates significantly different from saline group of same day ($P < 0.05$).

Table 1. Effect of paraquat pretreatment of rats on lung weight and perfusion pressure of isolated lungs*

Treatment	Days after treatment	Lung wet weight† (g)	Perfusion pressure† (mm Hg)	ΔPP†
Saline	2	1.49 ± 0.07	2.7 ± 0.1	0.0 ± 0.1
PQ	2	2.31 ± 0.39	2.3 ± 0.2‡	0.2 ± 0.2
Saline	3	1.40 ± 0.08	2.7 ± 0.1	0.2 ± 0.2
PQ	3	1.79 ± 0.14‡	2.6 ± 0.1	− 0.1 ± 0.1
Saline	4	1.95 ± 0.18	2.5 ± 0.9	0.1 ± 0.2
PQ	4	1.98 ± 0.29	2.8 ± 0.1	− 0.1 ± 0.1
Saline	10	1.47 ± 0.08	2.5 ± 0.1	− 0.1 ± 0.1
PQ	10	2.11 ± 0.25	2.6 ± 0.1	− 0.2 ± 0.0
Saline	17	1.87 ± 0.17	2.2 ± 0.1	0.0 ± 0.1
PQ	17	2.70 ± 0.42	2.1 ± 0.0	− 0.2 ± 0.0
Saline	28	1.77 ± 0.08	2.2 ± 0.2	− 0.2 ± 0.1
PQ	28	2.47 ± 0.65	2.1 ± 0.3	− 0.3 ± 0.1

* Rats were injected, i.p., with 25 mg/kg of paraquat dichloride (PQ) or with saline and their lungs were perfused 2–28 days later, as described in Materials and Methods.

† Results indicate post-perfusion lung weight, perfusion pressure at the onset of 5-HT perfusion, or the change in perfusion pressure (ΔPP) from the beginning to the end of the experimental period (final minus initial) and are expressed as means ± S.E.M. of groups of four to thirteen animals.

‡ Significantly different from saline-treated group ($P < 0.05$, two-tailed Student's *t*-test).

fraction to that in the homogenate was unaffected by pretreatment of rats with PQ. In lungs from untreated animals perfused for 5 min with medium containing 1 mM PQ and perfused with medium containing 1 mM PQ plus 0.1 μM [^{14}C]-5-HT, removal of the ^{14}C-amine did not differ from removal in lungs which were perfused with [^{14}C]-5-HT alone (data not shown). Thus, PQ does not directly inhibit 5-HT removal by lung. Similarly, concentrations of PQ up to 1 mM did not decrease monoamine oxidase activity in 600 *g* supernatant fractions of lung homogenates.

The effect of PQ pretreatment on the ability of lung to remove circulating angiotensin I was also examined. Clearance of perfused 1 ng/ml angiotensin I by lungs from PQ-pretreated rats did not differ from that of saline-pretreated controls (Table 4). As Table 5 indicates, however, angiotensin-converting enzyme activity in 600 *g* supernatant fractions of lung homogenates was depressed 4 days after PQ treatment. In this study, the ratio of ACE activity in the 600 *g* supernatant fraction of lung to that in the whole homogenate was not altered by PQ pretreatment. In addition, PQ, in concentrations up to 1 mM, did not directly inhibit ACE *in vitro*.

Table 2. Effect of paraquat pretreatment of rats on disposition of [^{14}C]-5-hydroxytryptamine by perfused lungs*

Treatment	Days after treatment	Per cent removal of 5HT† (% R)	Per cent of 5HT metabolized† (% M)	N
Saline	2	77.7 ± 4.0	26.1 ± 3.1	10
PQ	2	71.3 ± 3.2	26.9 ± 2.0	13
Saline	3	83.1 ± 1.8	35.2 ± 0.4	6
PQ	3	69.4 ± 4.0‡	30.2 ± 1.4‡	8
Saline	4	85.9 ± 1.4	30.8 ± 2.5	9
PQ	4	74.7 ± 2.0§	30.4 ± 1.6	11
Saline	10	83.4 ± 2.8	35.3 ± 0.8	6
PQ	10	86.5 ± 2.1	35.4 ± 2.6	8
Saline	17	89.9 ± 1.2	38.2 ± 0.9	7
PQ	17	89.9 ± 1.0	34.2 ± 1.7	8
Saline	28	74.2 ± 2.0	30.7 ± 1.7	4
PQ	28	73.7 ± 1.9	29.6 ± 1.4	4

* Rats were injected, i.p., with 25 mg/kg of paraquat dichloride (PQ) or with saline and their lungs were perfused 2–28 days later with 0.1 μM [^{14}C]-5-HT at 10 ml/min, as described in Materials and Methods. Results are expressed as means ± S.E.M.

† % R and % M were calculated as described in Materials and Methods.

‡ Significantly different from saline-treated groups ($P < 0.025$, two-tailed Student's *t*-test).

§ Significantly different from saline-treated group ($P < 0.01$, two-tailed Student's *t*-test).

Table 3. Effects of paraquat pretreatment on pulmonary monoamine oxidase (MAO) activity*

Treatment	Days after treatment	N	MAO activity (nmoles 5-HIAA/15 min) Per mg protein	Per g lung	Per whole lung
Saline	3	9	13.0 ± 1.3	1230 ± 95	1717 ± 67
PQ	3	9	8.3 ± 0.7†	686 ± 75†	1305 ± 74†
Saline	4	7	11.3 ± 0.8	1258 ± 70	1881 ± 58
PQ	4	15	11.0 ± 0.6	1065 ± 60	1685 ± 56‡

* Rats were injected i.p. with either 25 mg/kg of paraquat dichloride (PQ) or an equal volume of saline. MAO activity was measured, as described in Materials and Methods, using [^{14}C]-5-hydroxytryptamine as substrate.

† Significantly different from saline-treated group ($P < 0.005$, two tailed Student's t-test).

‡ Significantly different from saline-treated group ($P < 0.05$, two-tailed Student's t-test).

Table 4. Per cent of angiotensin I removed in a single pass by isolated perfused rat lung*

Days after treatment	Treatment Saline	Paraquat
2	61.9 ± 7.5	71.3 ± 6.6
3	81.9 ± 7.0	84.5 ± 6.1
4	70.0 ± 7.0	69.9 ± 5.4
10	76.4 ± 4.3	75.3 ± 8.7
17	70.7 ± 8.1	66.7 ± 7.6
28	81.7 ± 4.1	69.7 ± 4.8

* Rats were injected with 25 mg/kg of paraquat dichloride or with saline and their lungs were perfused 2–28 days later, as described in Materials and Methods. The number of animals per group ranged between four and thirteen. Results are expressed as means ± S.E.M.

DISCUSSION

A number of reports have appeared describing the morphology of lung following PQ administration to animals. Some investigators have described lesions of the pulmonary capillary endothelium following PQ administration to rats [3–5]. Others, however, have reported that alterations in endothelium either do not occur [6] or are so minor and infrequent that they cannot be construed as cellular injury [7]. In order to eludicate further the effect of PQ on pulmonary endothelium, we have examined metabolic parameters which are functions of pulmonary endothelial cells. For example, circulating 5-HT is taken into lung by a facilitated transport process which occurs in the pulmonary endothelium [16, 24, 25] and is then metabolized by pulmonary monoamine oxidase. Several factors may have influenced the observed decrease in 5-HT clearance occurring in perfused lungs from PQ-treated rats (Table 2). For example, shunting of perfusion medium may have contributed to this depression of 5-HT removal. If so, then a similar effect would be expected to occur with angiotensin I removal. Since no effect on angiotensin I removal was observed in these lungs (Table 4), it is unlikely that altered distribution of perfusion explains the observed effect on 5-HT clearance.

The impaired 5-HT clearance also could have been due to direct inhibition by PQ of the transport system for 5-HT or to inhibition of monoamine oxidase. Direct competitive inhibition of 5-HT uptake or of monoamine oxidase by residual PQ in lung 3 or 4 days after administration is unlikely since PQ is largely eliminated within 24 hr [35]. In addition, PQ in concentrations up to 1 mM did not inhibit MAO in lung homogenates. To test whether PQ directly inhibits pulmonary 5-HT removal, lungs from untreated rats were co-perfused with PQ and 5-HT. Since concentrations of PQ up to 1 mM in the perfusion medium failed to inhibit 5-HT removal, it is unlikely that effects observed in perfused lungs from PQ-treated rats occurred by this mechanism. Previous studies [16, 21] in perfused lung have shown that 5-HT clearance, measured shortly following initiation of amine perfusion, as in this study, is largely a function of facilitated uptake of the amine and not of its metabolism. Therefore, although pulmonary MAO activity was depressed by PQ pretreatment (Table 3), this probably does not account for the impaired removal of 5-HT by lungs of PQ-treated animals.

It is likely, therefore, that PQ inhibits pulmonary 5-HT clearance by interfering with the endothelial

Table 5. Effects of paraquat pretreatment on pulmonary angiotensin-converting enzyme (ACE) activity*

Treatment	Days after treatment	N	ACE activity (μmoles/min) Per g protein	Per g lung	Per whole lung
Saline	3	6	15.9 ± 0.7	2.26 ± 0.10	3.15 ± 0.14
PQ	3	9	17.3 ± 0.7	2.52 ± 0.09	3.77 ± 0.38
Saline	4	7	19.9 ± 1.6	1.77 ± 0.08	2.65 ± 0.06
PQ	4	15	13.9 ± 0.6†	1.20 ± 0.04†	1.91 ± 0.10†

* Rats were injected i.p. with either 25 mg/kg of paraquat dichloride (PQ) or an equal volume of saline. ACE activity was measured, as described in Materials and Methods, using HHL as substrate.

† Significantly different from saline treated group ($P < 0.001$, two-tailed Student's t-test).

transport process by which the amine is taken into lung. This could occur by at least two mechanisms. PQ treatment may damage a carrier molecule responsible for the transport of 5-HT across the endothelial cell membrane. Secondly, pulmonary 5-HT uptake appears to be an energy-requiring process [16, 21, 22, 28] which PQ may alter by decreasing energy pools such as intracellular ATP.

Other toxic agents which are known to produce extensive damage to pulmonary endothelium have been shown to alter the ability of lung to remove perfused 5-HT. For example, treatment of young rats with monocrotaline produces swelling and proliferation of pulmonary capillary endothelial cells and also decreases by 50 per cent the pulmonary clearance of 5-HT [26]. Similarly, Block and Fisher [27] have shown that exposure of rats to hyperoxia impairs 5-HT clearance by the perfused lung. Their finding that the metabolic function of lung was impaired before morphologic damage could be detected suggests that pulmonary 5-HT removal may be useful as a sensitive index of damage to pulmonary endothelium. Since morpholic damage to pulmonary endothelium has not been universally detectable following PQ administration, our finding of modest but statistically significant changes in metabolic indices associated with endothelium supports this view.

Lock *et al.* [36] have demonstrated that a number of primary amines including 5-HT inhibit the accumulation of PQ by lung slices. This finding raised the possibility that PQ and 5-HT accumulate in lung by the same mechanism and, therefore, may compete with each other for uptake. Later studies by these investigators [37] failed to support this possibility since the accumulation of these two compounds was affected differently in lung slices incubated in sodium-deficient medium or in lung slices from PQ-treated rats. Our observation that co-perfusion of lungs with up to 1 mM PQ did not affect 5-HT clearance also argues against the existence of a common transport mechanism.

The decreased uptake of 5-HT observed 3 days after PQ treatment likely led to a decreased intracellular 5-HT concentration. This decreased rate of substrate delivery to enzyme may have caused the diminished rate of 5-HIAA production by perfused lung (Table 2). However, MAO content of lung 3 days after PQ treatment was also depressed (Table 3), and this may have contributed to the depressed rate of metabolite formation in the perfusion studies. Witschi and Kacew [38] found depressed levels of MAO activity 24 hr after PQ, but only at doses more than twice that used in this study. Since MAO is bound to membranes of mitochondria, the decrease in activity of this enzyme may reflect ultrastructural alterations in mitochondria which occur in PQ poisoning [3–5, 7, 39].

Lung wet weight of PQ-treated rats was only slightly greater than that of controls (Table 1). This slight elevation in lung weight may have occurred either before or during perfusion. However, perfusion pressure, which invariably increases as lungs accumulate fluid during perfusion, did not increase in lungs from PQ-treated rats. This indicates that PQ pretreatment did not predispose lungs to edema formation during perfusion. The fact that the lung/body weight ratio of control rats that were not used in perfusion studies did not differ from that of similarly treated rats whose lungs were perfused suggests that perfusion *per se* did not injure the lungs. No correlation existed between lung weight and the magnitude of 5-HT clearance.

Despite careful attempts to maintain constant perfusion conditions, some treatment-independent variation occurred in both 5-HT and AI disposition from day to day. Accordingly, it is important when performing such studies that approximately equal numbers of control and treated lungs are perfused on a given day. Such protocol was carefully observed in this study.

Two phases of tissue response reportedly occur with toxicants which produce lung damage. The first phase involves cellular destruction by the toxicant and is followed by a reparative phase involving proliferation of endothelial cells and/or type II alveolar pneumocytes and replacement of damaged cells [40]. In this study, the earliest effects were not observed until more than 48 hr after PQ treatment. This agrees with the results of Smith *et al.* [37], who reported that treatment of rats with PQ 16 hr before being killed did not affect 5-HT accumulation by lung slices. The observed depression of pulmonary 5-HT disposition occurred at a time following PQ administration when most deaths occurred (Fig. 1) and also when the weight gain of the survivors was most severely retarded (Fig. 2). Only one animal died more than 4 days after PQ treatment, and surviving animals began to recover their initial loss in body weight between days 3 and 4 after paraquat administration. Similarly, effects of PQ pretreatment on pulmonary disposition of 5-HT returned to normal after day 4. These results similarly suggest two phases, an early phase of injury occurring maximally 3 days after treatment, followed by restoration of function occurring between 4 and 10 days after treatment. In the animals which succumbed, it is likely that PQ-induced damage to the parameters which we measured, as well as to other metabolic functions, was so severe that reparative processes were inadequate to restore function.

Pulmonary ACE has been localized to the luminal membrane of capillary endothelium [14]. Thus, alterations in ACE activity likely reflect changes in pulmonary endothelium. ACE activity in 600 *g* supernatant fractions of lung homogenates was depressed in lungs of rats treated 4 days previously with PQ (Table 5). This suggests that PQ damages this function of pulmonary endothelium and is in agreement with the effects of PQ administration on pulmonary 5-HT clearance (Table 2). No effect of PQ was observed, however, on the removal of angiotensin I by perfused lung (Table 4).

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