Biochemical Pharmacology, Vol. 44, No. 6, pp. 1029–1036, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

COMPETITION BETWEEN PARAQUAT AND PUTRESCINE FOR UPTAKE BY SUSPENSIONS OF RAT ALVEOLAR TYPE II CELLS

NIAN CHEN,* MARK R. BOWLES and SUSAN M. POND
University of Queensland Department of Medicine, Princess Alexandra Hospital, Brisbane, Queensland, Australia, 4012

(*Received* 27 *April* 1992; *accepted* 29 *June* 1992)

Abstract—Paraquat and the structurally similar polyamines, such as putrescine and spermidine, are accumulated actively and selectively by the alveolar type II cells via the polyamine uptake system. We report the uptake kinetics of paraquat and putrescine and their mutual inhibition in freshly isolated rat type II cell suspensions. The uptake of paraquat by type II cells exhibited saturation kinetics and could be inhibited in a concentration-dependent manner by putrescine. By applying enzyme kinetic analysis to our experimental data it was demonstrated that the uptake of paraquat or putrescine is inhibited in a partially competitive manner by the respective inhibitor. Thus, we postulate that the polyamine uptake pathway in type II cells for paraquat and putrescine has two separate sites, one for each substrate, and that binding of one leads to a conformational change in the other.

The lung is the target organ for paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride) toxicity because of the active accumulation of this herbicide by the alveolar type II cells. This uptake is mediated by the polyamine uptake system [1, 2]. Intracellular paraquat undergoes a well-described redox cycling which leads to the biochemical events that cause destruction of the type I and II cells [2, 3]. As demonstrated first by Rose *et al.* [1], the accumulation of paraquat into the lung is energy dependent and saturable and the Michaelis constants for the process have been reported for lung slices from several species [4].

Because the lung is composed of as many as 40 different cell types, of which type II cells account for only about 15% of the total cell population [5], investigators have turned to the examination of paraquat and polyamine uptake by relatively pure populations of these target cells. Active uptake of paraquat into suspensions of freshly isolated type II cells from the rat [6] and rabbit [7] has been observed but Michaelis constants were not measured. Recently, Oreffo *et al.* [8] reported the Michaelis constants for the uptake of putrescine by type II cells maintained in culture for 48 hr. This compound is thought to share the same uptake pathway as paraquat [9]. However, these investigators could not determine the K_m and V_{max} for paraquat directly "because of variable response at different concentrations of the herbicide".

The loss of differentiated function of type II cells in culture has been well documented [5]. Because of this and our interest in examining the effects of possible antidotes to paraquat toxicity in pure populations of type II cells, we used freshly isolated rat type II cells to study the uptake of paraquat. Here we report the Michaelis constants for the uptake of paraquat and putrescine by these cells in suspension and the kinetics of the inhibition of each substrate by the other.

MATERIALS AND METHODS

Chemicals. Radiolabelled [methyl-^{14}C]paraquat (101 mCi/mmol) was purchased from Cambridge Research Biochemicals (Billingham, U.K.) and [1,4-^{14}C]putrescine (90.4 mCi/mmol) from New England Nuclear (MA, U.S.A.). High purity elastase (EC 3.4.4.7) was obtained from the Elastin Products Co. (MI, U.S.A.), DNAse I from Calbiochem (CA, U.S.A.), and fetal calf serum and bovine serum albumin (BSA†) from Flow (Seven Hills, Australia). Silicone fluid (density = 1.03 g/mL) used in these experiments was a mixture of three parts of melting point bath oil (high temperature) from Sigma (M-9389; MO, U.S.A.) with two parts of 556 cosmetic grade fluid from Dow Corning (MI, U.S.A.). Sodium pentobarbitone was supplied by Abbott (Asquith, Australia) and sodium heparin by David Bull Laboratory (Mulgrave, Australia). All other chemicals used were analytical grade.

Phosphate-buffered saline (PBS)–Hepes buffer (pH 7.4) contained NaCl (140 mM), KCl (5 mM), Na_2HPO_4 (2.5 mM), Hepes (10 mM) and glucose (6 mM). PBS–Hepes buffer with EGTA (0.2 mM from Sigma, MO, U.S.A.) is referred to as solution I. Solution II consisted of PBS–Hepes buffer

* Corresponding author: Dr N. Chen, Department of Medicine, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Queensland, Australia, 4102. Tel. (61) 7 240 5343; FAX (61) 7 240 5399.

† Abbreviations: BSA, bovine serum albumin; IgG, immunoglobulin G; LDH, lactate dehydrogenase; PBS, phosphate-buffered saline; Tricine, *N*-tris(hydroxymethyl)-methylglycine.

with $CaCl_2$ (2 mM) and $MgSO_4$ (1.3 mM). *N*-Tris(hydroxymethyl)-methylglycine (Tricine) buffer (pH 7.4) contained NaCl (128 mM), KCl (4.74 mM), KH_2PO_4 (1.185 mM), glucose (6 mM) and Tricine (10 mM). The Percoll solution (density = 1.04 g/mL) was prepared by mixing 10.8 mL Percoll (Uppsala, Sweden) with 4 mL of 10 times concentrated PBS–Hepes (pH 7.4) and 25.2 mL H_2O.

Rat immunoglobulin G (IgG) was purified by caprylic acid precipitation of serum from normal Sprague–Dawley rats using the method of McKinney and Parkinson [10].

Animals. This study was approved by the University of Queensland Animal Ethics Committee. Female pathogen-free rats of Wistar species (180–250 g) were supplied by the Central Animal Breeding House of the University of Queensland, Australia and were allowed free access to food and water.

Isolation of alveolar type II cells. Petri dishes were coated (6 mL/dish) with rat IgG (0.5 mg/mL) in Tris buffer (50 mM, pH 9.5). These were kept at 4° overnight and on the following day brought to room temperature and washed five times with Tricine buffer. Three to nine rats were used for each experiment. Type II cells were prepared essentially according to the method of Dobbs *et al.* [11] but with the addition of a Percoll gradient centrifugation step described by Rannels *et al.* [12].

Rats were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (45 mg/kg) mixed with sodium heparin (3750 U/kg) and placed supine on an operating table. The trachea was cannulated with polyethylene tubing (2 mm) and the animal ventilated mechanically (16 breaths/min) with a Palmer respirator (London, U.K.). A thoracotomy was performed and the inferior vena cava isolated and cut. The pulmonary artery was cannulated with polyethylene tubing (2 mm) and connected to a Masterflex pump (Cole-Parmer, IL, U.S.A.) which was attached to a LP-1A motor drive (Amicon, MA, U.S.A.). A small incision was made in the left ventricle to allow the perfusion fluid to drain. The pulmonary circulation was perfused with 50 mL of solution II (37°) at a rate of 12 mL/min. Following perfusion, the lung–heart complex was dissected from the thorax, and the lung lavaged eight times with solution I (37°) and twice with solution II (37°). The lung was then digested by intratracheal instillation of 15 mL elastase solution (30 U/mL in solution II, 37°) over 17 min and minced with sharp scissors to pieces of about 1 mm^3 in a petri dish containing 10 mL fetal calf serum and 3 mg of DNAse I. This mixture was filtered through a stainless steel screen (20 mesh) and then through a Nytal nylon mesh (7 μm, Swiss Screens, Australia). The filtrate was centrifuged at 100 *g* for 10 min, the supernatant discarded and the erythrocytes were lysed by osmotic shock. The remaining cell suspension was dispensed carefully onto the 10 mL Percoll solution and gradient centrifugation was then performed at 400 *g* for 30 min at 4°. The supernatant was discarded and the cell pellet resuspended in Tricine buffer and distributed in the IgG-coated petri dishes (5 mL/dish). These were maintained at 37° for 1 hr, following which the dishes were tipped back and forth three times, the contents emptied into a 50 mL centrifuge tube and centrifuged at 100 *g* for 10 min. The supernatant was discarded and the cell pellet resuspended in Tricine buffer containing 1.5% BSA to give a cell density of 2×10^6 cells/mL. The cells were kept at 4° in an ice bath until required. Cell viability was determined by the exclusion of Trypan blue and leakage of lactate dehydrogenase (LDH) [13]. The purity of the population was determined by staining using the modified Papanicolaou method [14].

Uptake of paraquat and putrescine by type II cells. The uptake of paraquat was studied at extracellular concentrations of 10–100 μM (up to 5.05 μCi/mL) and concentrations of putrescine of 0–1 mM. The uptake of putrescine was studied at concentrations of 1–10 μM (up to 0.45 μCi/mL) and concentrations of paraquat of 0–2 mM. Each experiment point was determined from at least triplicate measurements. To obtain the concentration of the radiolabelled substrate in the uptake mixture, and to ensure that there was no loss to binding to the glassware, small weighed samples (20 μL) of each uptake mixture were taken, cells in the samples lysed by the addition of 200 μL of distilled H_2O and the radioactivity determined.

Immediately before use, the type II cells (0.96 mL cell suspension/test tube) were placed in a water bath at 37° for 10 min. The inhibitor or normal saline (in 20 μL) was then added and the radiolabelled substrate (in 20 μL) introduced 10 min later. Aliquot samples of the suspension (170 μL), taken at 10, 20, 30 and 40 min after the addition of the substrate, were pipetted into micro-centrifuge tubes containing 50 μL KOH (3 M) overlayed with 60 μL silicone fluid. Each tube was spun for 15 sec in an Eppendorf microfuge (Hamburg, Germany) at 16,000 *g* and then left at room temperature overnight. The next day, the tubes were cut at the level of the silicone oil layer and the contents of the KOH layer removed and neutralized with 75 μL HCl (2 M). The concentration of the radioisotope in these solutions was determined by liquid scintillation counting.

In preliminary studies, it was determined by measuring LDH release from the cells [13] that the optimal extracellular medium for the studies was Tricine buffer containing 1.5% BSA. The range of BSA concentrations tested was 0–3% and the other buffer studied, RPMI 1640–Hepes. The LDH release (mean ± SD, % of total) after 60 min incubation in the Tricine buffer plus 1.5% BSA was 7.73 ± 1.47% (N = 3) compared to 9.48 ± 0.023% (N = 3) in RPMI 1640–Hepes medium plus 1.5% BSA. The LDH release assay was also used to demonstrate that exposure of the cells for 50 min at 37° to paraquat concentrations of 0.5–4 mM was not cytotoxic. The LDH release (mean ± SD, % of total) after 50 min incubation in Tricine buffer plus 1.5% BSA with 4 mM paraquat was 7.60 ± 1.77% (N = 3) compared to 7.27 ± 0.82% (N = 3) with normal saline. There was no significant difference between the two ($P > 0.7$).

Calculation of uptake rates and kinetics. The accumulation of substrate by the cells was expressed as pmoles of substrate/10^6 cells. The uptake rate was estimated from the slope of the plot of pmoles substrate/10^6 cells *vs* time, determined by linear

regression analysis. The data were analysed according to Michaelis–Menten kinetics as described by Dixon and Webb [15].

RESULTS

Characteristics of the type II cells

Of the isolated cells, 94.13 ± 2.83% were identified by the modified Papanicolaou staining technique as alveolar type II cells. The number of type II cells obtained per rat was 9.01 ± 2.24 × 10^6 (N = 105). Cell viability for all preparations that were used in subsequent experiments exceeded 95%. The uptake studies were performed within 4 hr of isolation of the cells which were shown to retain viability (>95% by Trypan blue exclusion) over this time.

Uptake of paraquat and putrescine by the type II cells

The plots of the uptake of [^{14}C]paraquat and [^{14}C]putrescine *vs* time, shown in Fig. 1, were linear for 40 min. The uptake of either paraquat or putrescine over 40 min by the cells represented less than 0.0056% or 7.4% respectively, of that available in the extracellular fluid.

In Fig. 2 it can be seen that the uptake of paraquat was saturable. As shown in Lineweaver–Burk plots (Fig. 3), the uptake of paraquat was inhibited in a concentration-dependent manner by putrescine and *vice-versa*, and the inhibition was competitive. The K_m (affinity constant for the substrate) and V_{max} (maximum velocity of uptake) for uptake of paraquat were 88 μM and 2.85 pmol/10^6 cells/min, respectively. Similar studies with putrescine gave a K_m of 2.5 μM and a V_{max} of 3.33 pmol/10^6 cells/min. These results are presented in Table 1. As shown for paraquat uptake in Fig. 4, the inhibition was not complete, i.e. paraquat uptake failed to decrease to zero in the presence of the inhibitor. Similar results were obtained for putrescine in the present study (data not shown) and by Oreffo *et al.* [8]. If we define $K_{m\,app.}$ as the apparent K_m according to Eqn 1:

$$\frac{1}{v} = \frac{K_{m\,app.}}{V_{max}} \frac{1}{S} + \frac{1}{V_{max}} \tag{1}$$

partial competitive inhibition is also demonstrated when the data are plotted as $K_{m\,app.}/V_{max}$ *vs* the concentration of inhibitor (Fig. 5). The slope of both lines decreased as the concentration of inhibitor was increased. The model that accounts for these kinetics [15], shown in Fig. 6, can be described according to Eqn 2:

$$v = \frac{V_{max}}{1 + \dfrac{K_m\,(1 + I/K_i)}{S\,(1 + I/K_i')}} \tag{2}$$

where v is the uptake rate; S and I are the concentration of substrate and inhibitor, respectively; and K_i and K_i' describe the equilibrium association constant for the interaction between the inhibitor and the polyamine transport system alone, and the inhibitor and receptor–substrate complex, respectively.

To investigate the number of classes of binding site on the polyamine transport system, the data were analysed according to the Hill plot [15]:

$$\log \frac{v}{V_{max} - v} = n \log S + \log k \tag{3}$$

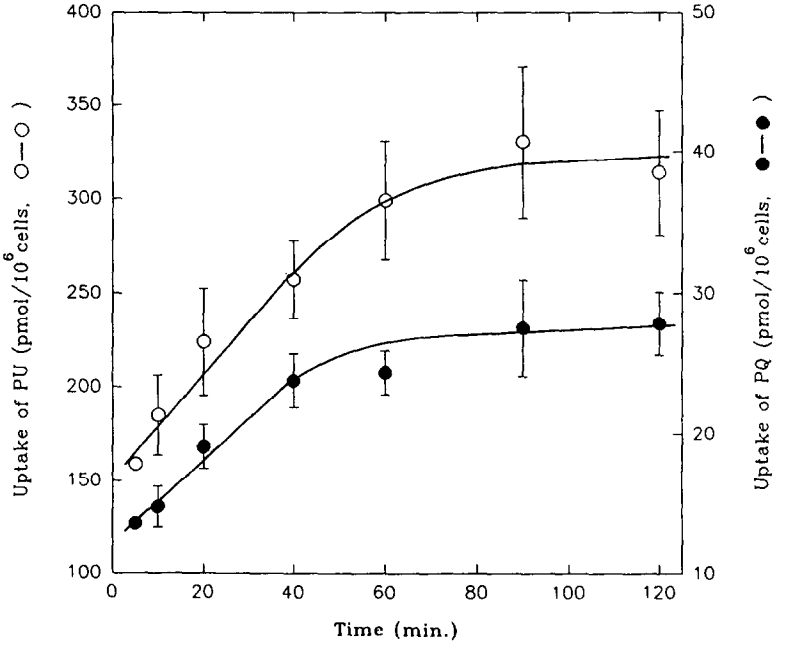

Fig. 1. Time course of uptake (mean ± SE) of paraquat (PQ) (●) (N = 5) and putrescine (PU) (○) (N = 4) by alveolar type II cells freshly isolated from rat lung.

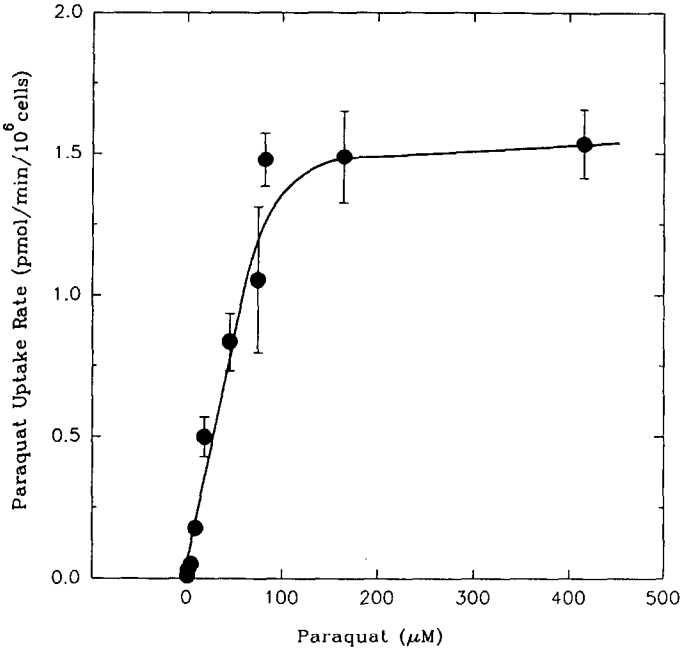

Fig. 2. Uptake rate of paraquat (mean ± SE, N = 3) at different concentrations of the substrate by alveolar type II cells freshly isolated from rat lung.

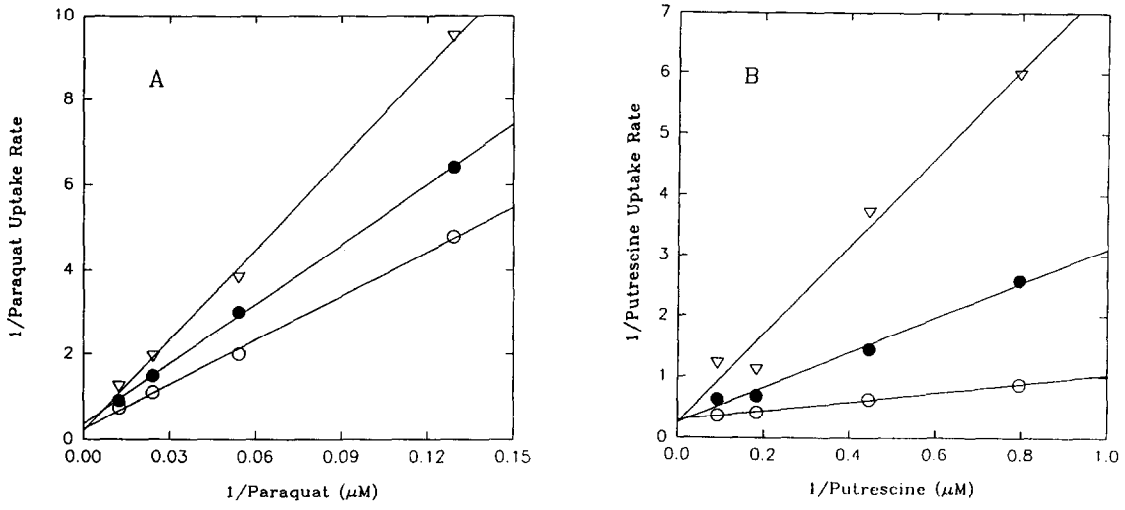

Fig. 3. (A) Lineweaver–Burk plot of paraquat uptake in the absence of inhibitor (○) or in the presence of putrescine, 0.5 μM (●) or 1.0 μM (▽) by alveolar type II cells freshly isolated from rat lung. (B) Lineweaver–Burk plot of putrescine uptake in the absence of inhibitor (○) or in the presence of paraquat, 250 μM (●) or 500 μM (▽) by alveolar type II cells.

Table 1. Kinetic constants for the uptake of paraquat and putrescine and their mutual inhibition in suspensions of type II cells freshly isolated from rat lung

Kinetic constant	Paraquat uptake	Putrescine uptake
K_m (μM)	87.98	2.46
K_i (μM)	1.64	86.31
K_i' (μM)	22.08	1010.89
K_m' (μM)	1187.75	28.78
V_{max} (pmol/min/10^6 cells)	2.85	3.33

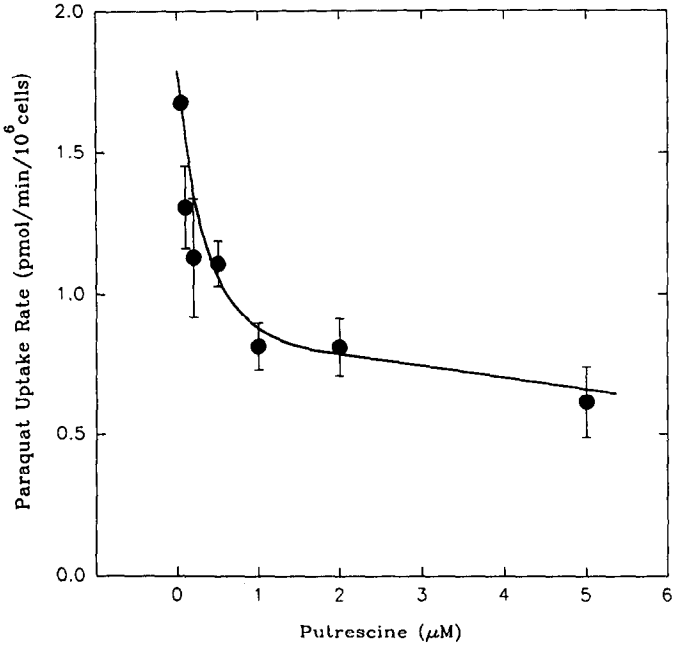

Fig. 4. Uptake rate (mean ± SE, N = 3) of paraquat (100 μM) by alveolar type II cells freshly isolated from rat lung in the presence of the inhibitor putrescine.

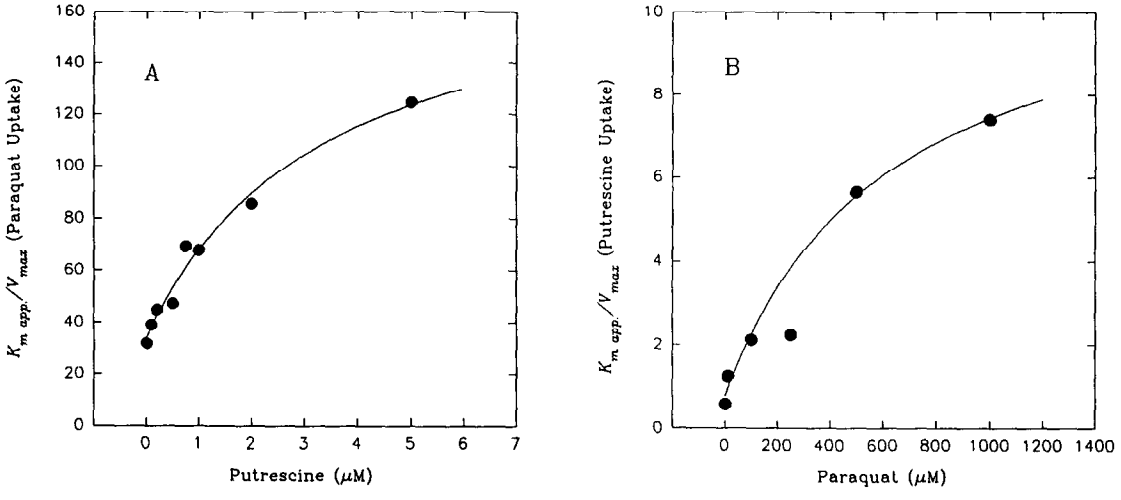

Fig. 5. Relationship between $K_{m\,app.}/V_{max}$ and the concentration of the respective inhibitor putrescine (A) and paraquat (B).

where v is the uptake rate of the substrate in the absence of inhibitor and n is the number of classes of binding site for the substrate. This number, determined by calculating the slope of the plot of $\log[v/(V_{max} - v)]$ *vs* $\log S$ (Fig. 7), was unity for both paraquat and putrescine.

DISCUSSION

We used two purification steps during the isolation of type II cells, gradient centrifugation and removal of macrophages and lymphocytes by "panning" in dishes coated with IgG. These were performed in order to obtain cell suspensions of high viability and homogeneity that could be used immediately for the uptake studies. However, this resulted in the yield of cells being lower than that reported by others [11].

The parameters of paraquat uptake demonstrated here by freshly isolated type II cells are consistent with those reported by others (Table 2). The uptake constants estimated by Smith and Wyatt [16] in rat lung slices (70 μM) and by Oreffo *et al.* [8] in cultured type II cells (69 μM) are similar to that reported in the current study. Karl and Friedman [17] obtained a higher K_m of 119 μM but used Sprague–Dawley

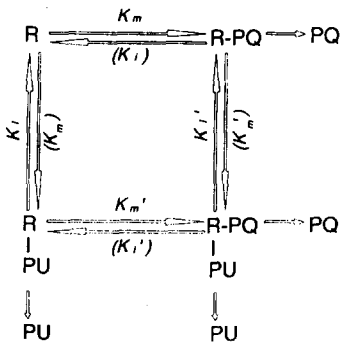

Fig. 6. Schematic representation of a functional model of the paraquat (PQ) and putrescine (PU) uptake pathways (parameters in parentheses refer to putrescine uptake and R represents the receptor).

rats rather than Wistar which could account for the difference. As observed in the lung slice study of Karl and Friedman [17], our cell suspension study demonstrated that the V_{max} values for paraquat uptake and putrescine uptake are similar.

Our results for the uptake of putrescine are also consistent with those reported by others (Table 2). Although we did not measure the DNA content of each cell preparation used in the current study, it was determined subsequently in similar preparations in our laboratory by the method of Cesarone *et al.* [18] to be 5.97 ± 1.24 μg DNA/10^6 cells (mean ± SD, N = 9). We used this value and the 4.14 μg protein/μg DNA suggested by Lewis [19] to convert some V_{max} values to the units of pmol/μM DNA/hr and enable comparisons between the various studies (Table 2).

In contrast to the difficulties experienced by some workers [8, 19], we have been able to determine the kinetics of paraquat uptake by type II cells successfully. This can probably be attributed to the preservation of function in freshly isolated suspensions of the cells in contrast to cultured cells. We also used [^{14}C]paraquat with a high specific activity (101 mCi/mmol) to obtain reliable uptake data.

Although it is assumed that paraquat and putrescine share the same polyamine uptake pathway(s) in the membrane of the type II cells [9], few studies have examined the mutual inhibition of these two substrates. Smith and Wyatt [16] demonstrated this inhibition in rat lung slices but did not derive any values for K_i or examine the nature of the interaction. Karl and Friedman [17] showed in their lung slice study that the affinity of the transport system was much greater for putrescine than for paraquat. They also suggested that paraquat and putrescine are accumulated by the same cell type. Recently, Oreffo *et al.* [8] reported that paraquat was a partial competitive inhibitor of the accumulation of putrescine by cultured type II cells. Our results for the inhibition of paraquat by putrescine are essentially the mirror image of theirs. The kinetic analyses used in the current study and by Oreffo *et al.* [8] are directly analogous to those used in enzyme kinetics and can be interpreted similarly. A fully competitive inhibition usually indicates that the inhibitor binds to the same site on the receptor as the substrate. However, in partially competitive inhibition, the substrate and inhibitor

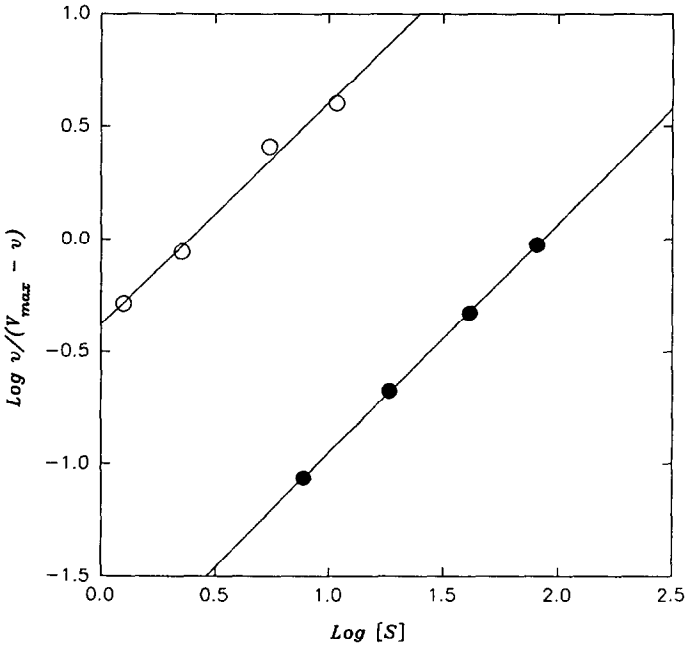

Fig. 7. Hill plots of log $[v/V_{max}\text{-}v]$ vs log S. The slope of the plot for paraquat uptake (●) is 1.019 (r = 0.9999) and for putrescine uptake (○), 0.988 (r = 0.9940).

Table 2. Kinetic constants for the uptake of paraquat and putrescine by various preparations from the lung reported in the current study and in the literature*

	Paraquat uptake		Putrescine uptake		Strain of rat	Reference
	K_m (μM)	V_{max}	K_m (μM)	V_{max}		
Lung slices			7	5.5‡	Wistar	[16]
			12–18		Wistar derived	[9]
			13.5	11.97‡	Wistar	[22]
			30.6	14.5‡	Wistar derived	[23]
	70	5‡			Wistar derived	[1,4]
	119	10.6‡	7.98	8.01‡	Sprague–Dawley	[17]
Cultured cells			5.9	17§	Wistar derived	[19]
			8.12	58§	CD	[24]
	29				Wistar	[25]
	64†		14.7	130§	CD	[8]
Cell Suspensions	88	28.64§	2.46	33.47§	Wistar	Current study

* The units of some values were converted as described in the text.
† The value was determined indirectly.
‡ Units are nmol/g lung/min.
§ Units are pmol/μM DNA/hr.

bind to different sites in such a way that the binding of either one causes a conformation change in the receptor. This decreases the ability of the receptor to bind the competing substrate. The observed similarity between the K_m for paraquat and its K_i for inhibition of putrescine uptake (Table 1; Fig. 6) must be due to rate-limiting transport of paraquat from the receptor into the cell. The corollary holds for the uptake of putrescine and its inhibition by paraquat. Assuming that $K_m/K'_m = K_i/K'_i$ [15], then K'_m can be calculated. These values, which are presented in Table 1, are also similar for the same reason.

Although it is probable that there are multiple pathways for the uptake of various polyamines by the type II and other cells [20, 21], the number of pathways involved in the uptake of paraquat has not been defined. On the basis of studies in Chinese hamster ovary cells, some of which were normal and others of which were deficient in the uptake pathway for paraquat and the polyamine methylglyoxal bis(guanylhydrazone), Byers *et al.* [20] suggested that paraquat was taken up by at least one but not by all of the polyamine uptake pathways. Our data and those of Orrefo *et al.* [8] lead us to reach a similar conclusion and to accept the most parsimonious uptake model, one which involves a single uptake pathway for paraquat by the type II cells.

Acknowledgements—This research was supported by the Australian National Health and Medical Research Council. We are most grateful to Miss Michelle McKellar for expert technical assistance and Mr Gus Nathan who assisted with the cytologic examinations.

REFERENCES

1. Rose MS, Smith LL and Wyatt I, Evidence for energy-dependent accumulation of paraquat into rat lung. *Nature* **252**: 314–315, 1974.
2. Smith LL, Lewis CPL, Wyatt I and Cohen GM, The importance of epithelial uptake systems in lung toxicity. *Environ Health Perspect* **85**: 25–30, 1990.
3. Smith LL, Mechanisms of paraquat toxicity in lung and its relevance to treatment *Hum Toxicol* **6**: 31–36, 1987.
4. Rose MS, Lock EA, Smith LL and Wyatt I, Paraquat accumulation: tissue and species specificity. *Biochem Pharmacol* **25**: 419–423, 1976.
5. Rannels SR and Rannels DE, Isolation and culture of alveolar type II cells for toxicologic studies. In: *Toxicology of the Lung* (Eds. Gardner DE, Crapo JD and Massaro EJ), pp. 219–238. Raven Press, New York, 1988.
6. Forman HJ, Aldrich TK, Posner MA and Fisher AB, Differential paraquat uptake and redox kinetics of rat granular pneumocytes and alveolar macrophages. *J Pharmacol Exp Ther* **221**: 428–433, 1982.
7. Horton JK, Brigelius R, Mason RP and Bend JR, Paraquat uptake into freshly isolated rabbit lung epithelial cells and its reduction to the paraquat radical under anaerobic conditions. *Mol Pharmacol* **29**: 484–488, 1986.
8. Oreffo VIC, John RA and Richards RJ, Diamine uptake by rat lung type II cells *in vitro*. *Biochem Pharmacol* **41**: 1209–1215, 1991.
9. O'Sullivan MC, Golding BT, Smith LL and Wyatt I, Molecular features necessary for the uptake of diamines and related compounds by the polyamine receptor of rat lung slices. *Biochem Pharmacol* **41**: 1839–1848, 1991.
10. McKinney MM and Parkinson A, A simple non-chromatographic procedure to purify immunoglobulins from serum and ascites. *J Immunol Methods* **96**: 271–278, 1987.
11. Dobbs LG, Gonzalez R and Williams M, An improved method for isolating type II cells in high yield and purity. *Am Rev Respir Dis* **134**: 141–145, 1986.
12. Rannels DE, Kamcji R, Pegg AE and Rannels SR, Spermidine uptake by type II pneumocytes: interactions of amine uptake pathways. *Am J Physiol* **257**: L346–L353, 1989.
13. Bergmeyer HU, *Methods of Enzymatic Analysis* (translated by Williamson DH); pp. 736–741. Academic Press, Verlag Chemie, 1965.
14. Kikkawa Y and Yoneda K, The type II epithelial cell of the lung, I. Method of isolation. *Lab Invest* **30**: 76–84, 1974.
15. Dixon M and Webb EC, *Enzymes*. Academic Press, New York, 1979.
16. Smith LL and Wyatt I, The accumulation of putrescine

into slices of rat lung and brain and its relationship to the accumulation of paraquat. *Biochem Pharmacol* **30**: 1053–1058, 1981.
17. Karl PI and Freidman PA, Competition between paraquat and putrescine for accumulation by rat lung slices. *Toxicology* **26**: 317–323, 1983.
18. Cesarone CF, Bolognesi C and Santi L, Improved microfluorometric DNA determination of biological material using 33258 Hoechst. *Anal Biochem* **100**: 188–197, 1979.
19. Lewis CPL, The pulmonary uptake and metabolism of cystamine. PhD Thesis, University of London, 1989.
20. Byers TL, Kameji R, Rannels DE and Pegg AE, Multiple pathways for uptake of paraquat, methylglyoxal bis(guanylhydrazone), and polyamines. *Am J Physiol* **252**: C663–C669, 1987.
21. Byers TL, Wechter R, Nuttal ME and Pegg AE, Expression of a human gene for polyamine transport in Chinese-hamster ovary cells *Biochem J* **263**: 745–752, 1989.
22. Hardwick SJ, Adam A, Smith LL and Cohen GM, Potentiation of the cell specific toxicity of paraquat by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). *Biochem Pharmacol* **39**: 581–589, 1990.
23. Nemery B, Smith LL and Aldrich WN, Putrescine and 5-hydroxytryptamine accumulation in rat lung slices: cellular localization and responses to cell-specific lung injury. *Toxicol Appl Pharmacol* **91**: 107–120, 1987.
24. Richards RJ, Davies N, Atkins J and Oreffo VIC, Isolation, biochemical characterization, and culture of lung type II cells of the rat. *Lung* **165**: 143–158, 1987.
25. Van der Wall NAA, Van Oirschot JFLM, van Dijk A, Verhoef J and Van Asbeck BS, Mechanism of protection of alveolar type II cells against paraquat-induced cytotoxicity by deferoxamine. *Biochem Pharmacol* **39**: 1665–1671, 1990.