traction, the latter by increasing the Ca^{2+}-flux into the cell [3, 8, 22, 23]. Moreover a combination of these two mechanisms could be considered as well.

In this context, it is noteworthy that several groups have reported the cyclic AMP induced phosphorylation of several different SL proteins *in vitro* [3, 21, 22, 24]. Although at present any physiological role of one or more of these SL membrane proteins remains to be established, we may now have an indication that more than one phosphorylateable SL protein is involved in the cyclic AMP dependent modulation of Ca^{2+}-fluxes across the SL.

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The accumulation of polyamines and paraquat by human peripheral lung

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The herbicide paraquat (1,1′-dimethyl-4,4′-bipyridylium) can accumulate in the lung of various species including humans [1, 2]. This accumulation has been shown to obey saturation kinetics and to be energy-dependent [2, 3]. The system responsible for the accumulation in rat lung is different from that reported for the uptake of the monoamine 5-hydroxytryptamine [4], although it may be the same as that responsible for the accumulation of the diamine putrescine [5]. Recently Smith *et al.* [6] also decribed the energy-dependent accumulation of the endogenous polyamines, spermine and spermidine, by rat lung slices, which appears to be similar to that previously described for the uptake of paraquat and putrescine [5].

The existence of systems for the accumulation of spermidine and spermine are not unique to the lung. Similar systems have been described in mouse and rabbit brain [7, 8] and human leukocytes [9]. In the mouse brain [7], the accumulation of spermidine and spermine is sodium-independent and appears to be similar to the accumulation of putrescine in rat lung and brain [5]. More recently two very high-affinity systems have also been described for the accumulation of spermine in rat brain slices [10] but these systems were sodium-dependent and therefore may be different from the sodium-independent system previously

Table 1. The accumulation of paraquat by human lung slices

Patient	Rate of accumulation of paraquat (10 μM) (nmoles/g wet wt lung/hr)	% inhibition of paraquat accumulation by Putrescine (10 μM)	Spermidine (10 μM)	Spermine (10 μM)
E	16	50	100	67
F	5	88	58	89
I	27	74	44	ND

Incubations were carried out at 37° in medium containing [^{14}C]paraquat and the accumulation of radioactivity was measured after 30, 60 and 120 min. The rates of accumulation of paraquat were subsequently determined by linear regression.
ND = not determined.

described for the uptake of paraquat [4] and putrescine [5] by rat lung slices.

Although paraquat has proved extremely safe in use, fatalities have resulted from the ingestion of the concentrated commercial solution. The symptoms of toxicity depend on the amount consumed but perhaps the most characteristic feature of paraquat poisoning is damage to the lung. This organ-specific toxicity can at least in part be explained by the selective accumulation of paraquat in the lung [1]. Thus the presence of an uptake system for the accumulation of putrescine in human lung could explain the accumulation of paraquat in man. In the present study, we have measured the ability of human peripheral lung to accumulate three endogenous polyamines (putrescine, spermidine and spermine) and also paraquat. We have shown that in the human lung there exists an energy-dependent system for the accumulation of polyamines and that it is this system which appears to be responsible for the pulmonary accumulation of paraquat *in vivo*.

Materials. [^{14}C]Methyl paraquat dichloride (30 mCi/mole), [1,4-^{14}C]putrescine dihydrochloride (116 mCi/mmole), [^{14}C]spermidine trihydrochloride (120 mCi/mmole) and [^{14}C]spermine tetrahydrochloride (122 mCi/mmole) were purchased from the Radiochemical Centre (Amersham, U.K.). Analytical-grade paraquat dichloride (99% pure) was a gift of ICI Plant Protection Division (Jealotts Hill, U.K.). Putrescine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride were obtained from the Sigma Chemical Co., and potassium cyanide from BDH Biochemicals Ltd. Leibovitz L-15 medium was obtained from Gibco Biocult Ltd (Paisley, U.K.).

Methods. Macroscopically normal peripheral lung specimens from lung cancer patients were obtained during surgery and were transported to the laboratory in Leibovitz L-15 medium with 2 mM L-glutamine at 4° and used within 2 hr. Samples were sliced (0.5 mm thick) using a McIlwain tissue chopper (Mickle Laboratories, Surrey, U.K.) and weighed into 50 mg lots. Accumulation of [^{14}C]paraquat, [^{14}C]putrescine, [^{14}C]spermidine or [^{14}C]spermine was carried out at 37 or 4°, in medium containing KCl (5.4 mM), $MgCl_2$ (1 mM), NaCl (111 mM), NaH_2PO_4 (9.6 mM), HEPES (2.5 mM) and glucose (11 mM) (pH 7.4) essentially as described by Smith and Wyatt [5]. Incubations were carried out in 3 ml of medium containing the appropriate concn of unlabelled chemical and 0.1 μCi of the required ^{14}C-labelled chemical. The accumulation of paraquat was measured after 30, 60 and 120 min of incubation and that of the polyamines after 15, 30 or 60 min. After incubation, tissues and media were treated as described by Smith and Wyatt [5] and radioactivity determined by liquid scintillation spectrometry. The radioactivity was used as a measure of the presence of each compound. Although no data is available for human lung we have shown this to be acceptable in rat lung tissue [6].

Results. The ability of slices of human lung to accumulate the polyamines putrescine, spermidine and spermine or paraquat was variable compared with the results generated in our laboratories using rat lung slices. The variation was not confined to accumulation *per se* but also to the inhibition studies. This problem can probably be explained, at least in part, by the use of lung tissue from various individual patients of differing age with a differing background pathology in their lung. Also the conditions of study varied due to the handling of the tissue and the transport of the tissue to our laboratory. We have chosen not to select data for presentation and have included data which demonstrates the variability of the results. Despite this, we consider that the interpretation of the data reflects the weight of evidence.

Paraquat (10 μM) was accumulated into human lung in a linear, time-dependent manner, in agreement with Smith *et al.* [2], although the rate of accumulation varied between patients [mean of 16 ± 6 nmoles of paraquat/g wet wt lung/hr (N = 3, ± S.E.)]. The accumulation was markedly inhibited by the presence of an equimolar concn of any of the naturally occurring polyamines studied (i.e. putrescine, spermidine or spermine) (Table 1).

Putrescine (10 μM) was accumulated by human lung slices, in a time-dependent manner, at a rate of 77 ± 14 nmoles/g wet wt lung/hr (mean ± S.E., N = 12) (Table 2). The pulmonary uptake of putrescine was significantly reduced at 4° (lung slices from patients L and M showed 99 and 98% inhibition respectively). In the presence of potassium cyanide (1 mM) lung slices from patients L and M showed 54 and 72% inhibition respectively. These results suggest that the accumulation was energy-dependent. Accumulation of putrescine (10 μM) was inhibited 44–100% by the presence of an equimolar concn of either spermine or spermidine (Table 2). Paraquat (10 μM) also inhibited putrescine accumulation into lung slices although it was considerably less effective than spermine or spermidine (Table 2).

Spermidine (10 μM) was accumulated by lung slices at a rate of 37 ± 5 nmoles/g wet wt lung/hr (mean ± S.E., N = 6) (Table 3). The accumulation was also markedly inhibited at 4° (lung slices from patients L and M showed 100 and 64% inhibition respectively) and partially inhibited by potassium cyanide (1 mM) (lung slices from patients L and M showed 34 and 53% inhibition respectively). This suggested that the uptake was energy-dependent. The accumulation of spermidine (10 μM) was partially reduced by an equimolar concn of putrescine but spermine (10 μM) had no effect (Table 3). However, at higher concns of either putrescine or spermine (100 μM), the accumulation of spermidine (10 μM) was almost totally inhibited (data not shown). The apparent lack of an inhibitory effect of the lower concn of spermine (10 μM) on the uptake of spermidine may be due to a lower affinity for the uptake system although given the paucity of data together with the variation in results we cannot be categoric.

The accumulation of spermine (10 μM) was measured in

Table 2. The accumulation of putrescine by human lung slices

Patient*	Rate of accumulation of putrescine (10 μM) (nmoles/g wet wt lung/hr)	% inhibition of putrescine accumulation by		
		Spermidine (10 μM)	Spermine (10 μM)	Paraquat (10 μM)
E	114	92	ND	31
F	27	100	44	0
G	116	62	61	12
H	43	63	ND	ND
I	68	63	76	ND
J	28	54	45	ND

* The rates of accumulation of putrescine (10 μM) were also determined in six other samples (patient A—84, B—160, C—145, K—28, L—82, M—23 nmoles putrescine/g wet wt lung/hr).

Incubations were carried out at 37° in medium containing [^{14}C]putrescine and the accumulation of radioactivity was measured after 15, 30 and 60 min. The rates of accumulation of putrescine were subsequently determined by linear regression.

ND = not determined.

Table 3. The accumulation of spermidine by human lung slices

Patient*	Rate of accumulation of spermidine (10 μM) (nmoles/g wet wt lung/hr)	% inhibition of spermidine accumulation by	
		Putrescine (10 μM)	Spermine (10 μM)
H	33	31	ND
I	37	16	0
J	38	12	7

* The rates of spermidine accumulation were also determined in samples from three other patients (patient K—44, I—53, M—19 nmoles spermidine/g wet wt lung/hr).

Incubations were carried out at 37° in medium containing [^{14}C]spermidine and the accumulation of radioactivity was measured after 15, 30 and 60 min. The rate of accumulation of spermidine was subsequently determined by linear regression.

ND = not determined.

Table 4. The accumulation of putrescine (10 μM–1 mM) into human lung slices

Patient	Rate of accumulation of putrescine (nmoles/g/hr) at media concns of				Apparent K_m (μM)	V_{max} (nmoles/g wet wt/hr)
	10 μM	20 μM	100 μM	1 mM		
A	98	203	160	461	11	172
B	160	165	237	332	9	249
C	145	163	171	169	2	169
E	44	76	90	481	9	99

Slices of human lung were incubated at 37° in medium containing various concentrations of [^{14}C]putrescine. The amount of putrescine in the slice was measured after 15, 30 and 60 min and from this the rate of accumulation was determined. The apparent K_m and V_{max} values were obtained from Woolf plots of ($\frac{[\text{putrescine in medium}]}{\text{uptake rate}}$ against [putrescine] in the medium) and were calculated over the linear range.

three patients (patient J—61, K—48, L—87 nmoles/g wet wt lung/hr). The uptake was also found to be energy-dependent being inhibited at 4° (lung slices from patients K and L showed 49 and 94% inhibition respectively) or by potassium cyanide (1 mM) (lung slices from patient K showed 94% inhibition). By extrapolation of the accumulation data to the zero time point it was apparent that there was considerably more spermine associated with the tissue than putrescine or spermidine. In one patient (J) where sufficient tissue was available, equimolar concns of either spermidine or putrescine inhibited spermine (10 μM) accumulation by 93 and 49% respectively. The inhibition

was concn-dependent in that higher concns of spermidine and putrescine had a significantly greater effect upon spermine uptake.

In order to further characterise the pulmonary uptake system of putrescine, a range of concns (10 μM–1 mM) of the diamine was incubated with lung slices (Table 4). A Lineweaver–Burk double-reciprocal plot of putrescine concn against the rate of accumulation of putrescine indicated that Michaelis–Menten kinetics were directly applicable in only one case (patient C). In the other three cases, the rates of accumulation at the highest substrate concn of putrescine (1 mM) were unexpectedly large. These results when expressed in the form of a Woolf plot (s/v against S) showed biphasic kinetics, being linear below a substrate concn of 100 μM. From these data it was possible to calculate apparent K_m values of 2–11 μM and V_{max} values of 99–249 nmoles of putrescine/g wet wt lung/hr for uptake of putrescine (using the range of putrescine concns of 10–100 μM. The high rates of uptake of putrescine observed at higher substrate concns (1 mM) (patients A, B and E) suggest that, despite saturation of the primary transport system, it may be possible to derive a second apparent K_m for the accumulation of putrescine. This would be a low-affinity system and with the limited tissue available it was not possible to derive a further kinetic constant. This possibility requires further investigation.

Discussion. The pulmonary toxicity of the herbicide paraquat is due at least in part to its accumulation in the lung. Smith and Wyatt [5] have recently reported that the same uptake process in the lung is responsible for the accumulation of both putrescine and paraquat. The present study indicates that a similar mechanism for the accumulation of putrescine also exists in human peripheral lung. Despite the variation in the effect of KCN this appears to be an energy-dependent process which may be inhibited by the presence of other naturally occurring polyamines. The inability of paraquat to cause a similar degree of inhibition (Table 2) may indicate that, as in the rat [5], the affinity of the system for putrescine is greater than for paraquat. The ability of putrescine to inhibit the accumulation of paraquat into human lung, further supports the view that the herbicide is accumulated into the lung by the same process as putrescine.

We have also demonstrated that two other naturally occurring polyamines, spermidine and spermine, are also accumulated into the human lung in an energy-dependent fashion. The mutual inhibition of polyamine uptake by other polyamines (Tables 2 and 3) suggests that they share a common uptake system. It therefore appears that the energy-dependent accumulation of polyamines into human lung slices is comparable to the system described in rat brain and lung [5].

A large inter-individual variation was observed in the pulmonary uptake by human lung of paraquat and the endogenous polyamines (Tables 1–3). A similarly large variability has been noted in other studies using human respiratory tissues, such as the ability to metabolize benzo[*a*]pyrene [11] or the binding of this carcinogen to DNA [12]. Due to this large inter-individual variability results of individual patients have generally been given. However, despite this variability it is our conclusion that in human lung, as well as rat lung, there exists an energy-dependent system for the accumulation of endogenous polyamines.

The reason(s) for the accumulation of polyamines in lung, brain or leukocytes is unclear. The physiological function of these compounds has been linked to many processes including tissue growth and regeneration [13, 14]. However, further experiments are required to determine whether these uptake systems are involved in the regulation or control of such processes in the lung.

In summary, human peripheral lung slices have been shown to accumulate paraquat, putrescine, spermidine and spermine. This accumulation was reduced at 4° and inhibited in the presence of potassium cyanide indicating that the uptake was energy-dependent. The accumulation of putrescine obeyed saturation kinetics and gave values for apparent K_m of 2–11 μM and V_{max} of 99–249 nmoles/g wet wt lung/hr. The three endogenous polyamines were mutually inhibitory suggesting that they share a common uptake system. Each of these polyamines inhibited the accumulation of paraquat into lung slices. Although paraquat was able to inhibit the accumulation of putrescine into human lung slices, it was less effective than either spermidine or spermine. These findings indicate that human lung has an uptake system for endogenous polyamines, similar to that previously described in rat lung, and that it is this system which is responsible for the accumulation of paraquat.

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