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# THE ACCUMULATION OF CYSTAMINE AND ITS METABOLISM TO TAURINE IN RAT LUNG SLICES

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**Abstract**—The objective of these studies was to determine the accumulation and fate of the disulphide, cystamine by rat lung slices. Cystamine was accumulated by two active uptake systems that obeyed saturation kinetics, with apparent $K_m$ values of 12 and 503 $\mu$M, and maximal rates of 530 and 5900 nmol/g wet weight/hr respectively. The high affinity system was competitively inhibited by the diamine, putrescine and the herbicide paraquat, which are themselves accumulated. Thus, this pulmonary uptake process appears to be identical for all three compounds. In contrast, the low affinity process was not inhibited by putrescine, and this process results from the diffusion of cystamine into the cell and its subsequent metabolism. Upon accumulation, cystamine was metabolised, predominantly to the sulphonic acid, taurine, with 10–20% of the intracellular label covalently binding to protein. Conversion to taurine was unaffected by amine oxidase inhibitors, but was decreased after GSH depletion, suggesting that pulmonary cystamine metabolism is glutathione-dependent, and is not mediated by diamine oxidase.

Both cystamine and taurine have been implicated as antioxidants, and we suggest that cystamine is actively accumulated by the lung as part of the process to protect pulmonary tissue against oxidative stress.

It is well established that the lung represents the primary target organ of paraquat (1,1′-dimethyl-4,4′-bipyridilium) toxicity [1–4]. It has been suggested that this is due to the presence of a saturable and energy-dependent uptake system specific to the lung, that is responsible for the selective accumulation of paraquat by this organ [5, 6].

The observation that the polyamines putrescine, spermidine and spermine are accumulated by the lung in a manner similar to paraquat [7, 8], coupled with evidence that each is able to inhibit the uptake of the other [9, 10], has led to the proposal that both paraquat and the polyamines enter the lung via a single system for which the polyamines are the endogenous substrates [7, 11]. Paraquat is thought to be able to utilise the system through its compliance with the structural requirements necessary for transport [12]. Consequently, paraquat is accumulated by the lung to a concentration several-fold greater than that in the plasma, or in other organs [6].

Studies in this laboratory have shown that the physiologically occurring thiol cysteamine (2-mercaptoethylamine) is able to inhibit competitively the uptake of paraquat into lung slices (unpublished observations), suggesting that this compound may also be transported into the lung by the polyamine uptake system. However, under physiological conditions, cysteamine and its disulphide cystamine (2,2′-dithio-bis[ethylamine]) exist in an equilibrium, which depends on the oxidative state of the environment. Intracellular conditions favour the formation of the sulphydryl, whilst in the plasma or extracellular fluid, the equilibrium is shifted towards the disulphide [13]. Moreover, since the structure of cystamine rather than cysteamine obeys the criteria defined for transport by this uptake system [12], we have suggested that cysteamine may undergo autooxidation in physiological media and consequently be delivered to the lung slice as the disulphide, in which form it is able to inhibit the uptake of paraquat.

In this study, therefore, we have attempted to determine whether the uptake system previously defined for paraquat and polyamine uptake is also able to facilitate the pulmonary accumulation of cysteamine/cystamine, and if so, what is the fate of cysteamine/cystamine subsequent to its accumulation.

## MATERIALS AND METHODS

### *Materials*

[U-$^{14}$C]Cystamine dihydrochloride (25 mCi/mmol) was purchased from Organics Division, ICI Ltd. (Billingham, U.K.). [1,4-$^{14}$C]Putrescine dihydrochloride (116 mCi/mmol) and [methyl-$^{14}$C]paraquat dichloride (111 mCi/mmol) were purchased from Amersham International Ltd. (Amersham, U.K.). Cystamine dihydrochloride, putrescine dihydrochloride, taurine, hypotaurine, aminoguanidine, diethylmaleate, dithiothreitol and

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5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. Ltd. (Poole, U.K.). Dihydroxyethyldisulphide (DHED) was purchased from Aldrich Chemical Co. Ltd. (Gillingham, Dorset, U.K.). Paraquat dichloride was provided by Plant Protection Division, ICI Ltd. (Surrey, U.K.). KCN and methyl cellulose TLC plates (Merck, Darmstadt, F.R.G.) were purchased from BDH Ltd. (Poole, U.K.). Soluene 350 (a tissue solubiliser) and Dimilume scintillation fluid were purchased from Packard Ltd. (Poole, U.K.). Optiphase MP scintillation fluid was purchased from FSA Laboratory Supplies Ltd. (Loughborough, U.K.) and halothane (Fluothane) was obtained from Pharmaceuticals Division, ICI Ltd. (Macclesfield, U.K.).

*Methods*

*Determination of the stability of cysteamine in incubation medium.* Cysteamine (1 mM), dithiothreitol (DTT, 1 mM) or cysteamine (1 mM) plus DTT (1 mM) were incubated in modified Krebs Ringer Phosphate (KRP) containing NaCl (130 mM), KCl (5.2 mM), $CaCl_2$ (1.9 mM), $MgSO_4$ (1.29 mM), $Na_2HPO_4$ (10 mM) and glucose (11 mM). The pH of the buffer was adjusted to 7.4. After incubation in a shaking water bath at 37° and 140 strokes/min, 1 ml medium was added to 1 ml 10% (w/v) trichloroacetic acid (TCA) on ice, and the non-protein sulphydryl content was determined by the method of Sedlak and Lindsay [14]. Since DTT also contains thiol groups, the level of thiol due to cysteamine in the presence of DTT was calculated by subtracting the levels of thiol associated with DTT found after incubating DTT alone.

*Preparation of lung slices.* Male Alpk : AP (Wistar-derived) specific pathogen free rats (body weights approximately 200 g) were killed by inhalation of an overdose of halothane, the lungs removed, and slices 0.5 mm thick prepared using a McIlwain tissue chopper.

*Inhibition of paraquat accumulation.* Freshly prepared lung slices (20–40 mg) were placed in 3.0 ml KRP containing 10 μM [$^{14}$C]paraquat in the presence or absence of 100 μM cysteamine, 1 mM DTT, 1 mM DTT plus 100 μM cysteamine or 100 μM cystamine. Incubations were carried out in a shaking water bath at 37° and 140 strokes/min for 60 min. The uptake of paraquat was determined as described below.

*Uptake of putrescine and cystamine into lung slices.* Lung slices were incubated in 3.0 ml KRP containing [$^{14}$C]cystamine or [$^{14}$C]putrescine (0.1 μCi) at 37° (unless stated) under air in a shaking water bath at 140 strokes/min.

The ability of putrescine to inhibit the uptake of [$^{14}$C]cystamine was determined after 10 min of incubation, over which time the uptake of the cystamine used was found to be linear. The effect of cystamine on the uptake of [$^{14}$C]putrescine was determined after 30 min of incubation, over which time the uptake of label was also linear.

To determine the effects of other treatments on the uptake or metabolism of cystamine, slices were incubated for 15 min under the appropriate conditions prior to the addition of [$^{14}$C]cystamine.

*Determination of the radioactivity in lung slices and medium.* Tissue slices were removed from the incubation medium, carefully blotted and washed with 2 × 1 ml KRP. They were then dissolved in 1 ml Soluene 350, and 10 ml Dimilume was added. Radioactivity was determined by liquid scintillation spectrometry. Also, an aliquot of medium (0.1 ml) was made up to 1 ml with water, 10 ml Optiphase MP added, and radioactivity was measured. The slice to medium ratio was calculated as the ratio of $^{14}$C present per unit weight of slice to the $^{14}$C present in an equal volume of medium. From this, the concentration of cystamine, paraquat or putrescine present in the slice was calculated.

*Determination of [$^{14}$C]cystamine and its metabolites in lung slice and medium.* Lung slices (30–50 mg) were incubated as described above, the tissue was removed into 1 ml 5% (w/v) TCA on ice, homogenised and left at 4° for 30 min with occasional stirring. The homogenate was centrifuged (20 min, 4°, 3000 *g*) and the supernatant fraction removed. An aliquot (0.1 ml) of this was added to 0.9 ml water, and the radioactivity present was determined. The radioactivity present in an aliquot (0.1 ml) of the incubation medium was determined in a similar manner.

The compounds associated with radioactivity in the lung supernatant fraction and the incubation medium were determined by thin layer chromatography. Twenty microlitres of supernatant or 10 μl of medium were spotted onto methyl-cellulose TLC plates along with a further 1.5 μl of a carrier solution of cystamine, taurine and hypotaurine (all at 33 mg/ml). [$^{14}$C]Cystamine and [$^{14}$C]taurine standards were also spotted on each plate. The plates were run in a solvent system of ethanol/water/ammonia (80/16/4) or phenol/water (100/39 w/v).

A quantitative determination of the separation of the radioactive compounds on the plate was made using an Isomess IM3000 radio TLC scanner. By this method, the percentage contribution of each separate peak to the total radioactivity on the track was determined. By calculating the total radioactive content of the lung slice by liquid scintillation spectrometry the absolute concentration of [$^{14}$C]cystamine and its metabolites was then estimated.

Levels of radiolabeled compounds in the incubation medium were determined by measuring the radioactivity associated with a known concentration of [$^{14}$C]cystamine in the absence of tissue, and measuring the fraction of that radioactivity remaining after incubation with lung slices. The total radioactivity present was then translated to a concentration of [$^{14}$C]cystamine equivalents, and the percentage contribution of each peak on the chromatogram to the total radioactivity could then be converted to an absolute concentration in [$^{14}$C]cystamine equivalents.

*Measurement of NPSH levels in lung slices.* Non-protein sulphydryl levels in the lung slices were measured using the method of Sedlak and Lindsay [14]. Briefly, the tissue was homogenised in 5% (w/v) TCA, and centrifuged (4°, 3600 rpm, 20 min). An aliquot of the supernatant fraction in Tris buffer (pH 8.9) was treated with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and the absorbance at 412 nm measured.

*Statistical analysis.* Statistical analysis was performed using the Student's *t*-test for paired data. Significance was selected at $P < 0.05$.

## RESULTS

### *Stability of cysteamine in incubation medium*

In KRP buffer at pH 7.4, cysteamine-associated thiol levels were rapidly depleted, with a 90% loss occurring within 15 min (Fig. 1). However, in the presence of 1 mM DTT, cysteamine-associated thiol levels were maintained at approximately 80% of control over 30 min.

### *Inhibition of paraquat uptake by cysteamine*

The addition of cysteamine (100 $\mu$M) produced a marked (70%) inhibition in the uptake of 10 $\mu$M paraquat. However, this effect was completely abolished in the presence of 1 mM DTT (the latter having no significant effect on uptake in the absence of cysteamine). The disulphide cystamine, also at a concentration of 100 $\mu$M, reduced paraquat uptake to 20% of control, this being a significantly greater effect than that of its corresponding thiol (Fig. 2).

In subsequent studies, the uptake of the disulphide, cystamine by lung slices was investigated, since our data suggest that cysteamine is rapidly oxidised under our experimental conditions, and that only as the disulphide is it able to interact with the paraquat uptake system.

### *Cystamine accumulation*

The uptake of radioactivity from [$^{14}$C]cystamine by lung slices was found to be linear with time over 5 min at all concentrations used. At concentrations up to 30 $\mu$M, this linearity was maintained over a further 5 min. The initial rate of uptake was therefore calculated over 10 min for concentrations below 100 $\mu$M, and over 5 min only for concentrations in excess of 100 $\mu$M.

The initial rate of uptake of radiolabel exhibited a biphasic relationship to the external [$^{14}$C]cystamine concentration. Both components obeyed saturation kinetics and exhibited apparent $K_m$ values of 12 and 503 $\mu$M, and maximal rates of 530 and 5900 nmol/g wet weight/hr respectively, as defined by a Hanes–Woolf plot fitted by linear regression (Fig. 3).

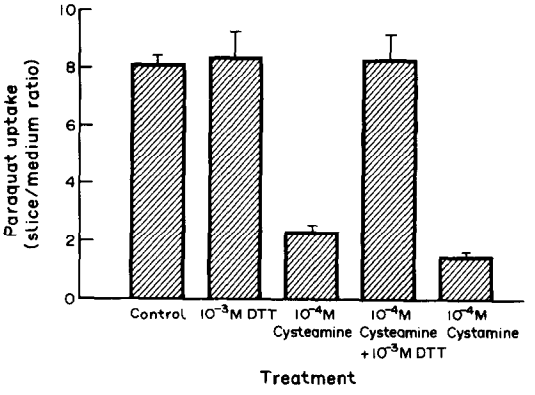



Fig. 2. Effect of the addition of cysteamine or cystamine to the incubation medium on the uptake of [$^{14}$C]paraquat by rat lung slices. Slices of rat lung were incubated at 37° in KRP glucose medium containing 10 $\mu$M [$^{14}$C]paraquat in the presence or absence of cysteamine (100 $\mu$M), cysteamine (100 $\mu$M) + DTT (1 mM) or cystamine (100 $\mu$M). The accumulation of $^{14}$C-label was measured after 60 min using radiochemical techniques. The results are expressed as the means ± SEM of three observations.

### *Putrescine inhibition of cystamine uptake*

The uptake of radioactivity from [$^{14}$C]putrescine by lung slices was inhibited by cystamine (25–200 $\mu$M) in a concentration-dependent manner. Moreover, the mode of inhibition was shown to be competitive (Fig. 4a). An inhibition constant ($K_i$) of 11 $\mu$M was obtained by a Dixon plot.

The uptake of [$^{14}$C]cystamine at concentrations below 10 $\mu$M was competitively inhibited by putrescine (50 $\mu$M). However, subsequent increases in the putrescine concentration up to 200 $\mu$M resulted in little or no further inhibition, a significant residual

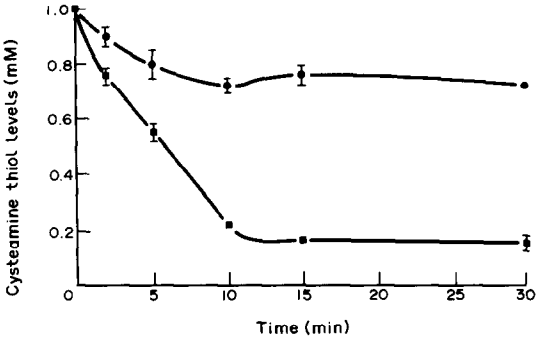



Fig. 1. Stability of cysteamine in Krebs–Ringer phosphate buffer. Cysteamine (1 mM) was incubated at 37° in the presence or absence of the reducing agent dithiothreitol (DTT; 1 mM). Cysteamine-associated non-protein sulphydryl levels were followed over a time course by derivatisation with DTNB and measuring the absorbance at 412 nm. ■—■, cysteamine (1 mM); ●—●, cysteamine (1 mM) + DTT (1 mM). The results are expressed as the means ± SEM of three observations.

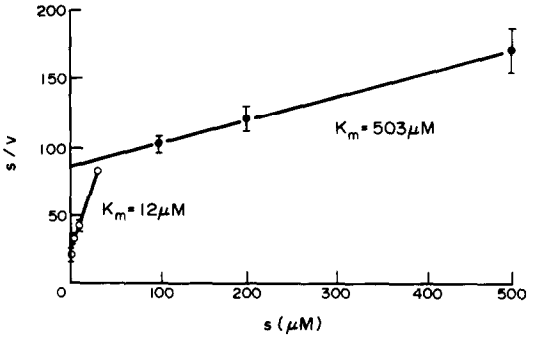



Fig. 3. Hanes–Woolf plot of [$^{14}$C]cystamine uptake by rat lung slices. Slices of rat lung were incubated in KRP glucose medium containing various concentrations of [$^{14}$C]cystamine. The amount of $^{14}$C-radiolabel accumulated was measured after 5 and 10 min by radiochemical techniques. The results are expressed as the means ± SEM with eight animals for each observation.

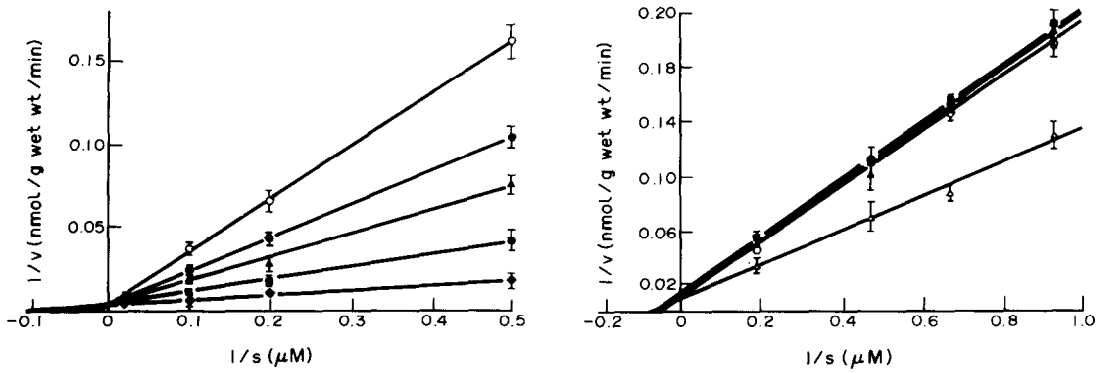



Fig. 4. (a) Lineweaver–Burk plot describing the inhibition of [$^{14}$C]putrescine uptake by cystamine. Slices of rat lung were incubated at 37° in KRP glucose medium containing various concentrations of [$^{14}$C]putrescine and cystamine. The accumulation of $^{14}$C-label was measured after 30 min by radiochemical techniques. The results are expressed as the mean ± SEM with four animals for each observation. ◆—◆ control; ■—■ 25 μM; ▲—▲ 50 μM; ●—● 100 μM; ○—○ 200 μM cystamine. (b) Lineweaver–Burk plot describing the inhibition of [$^{14}$C]cystamine uptake by putrescine. Slices of rat lung were incubated in KRP glucose medium containing various concentrations of [$^{14}$C]cystamine and putrescine. The amount of $^{14}$C-label accumulated was measured after 10 min by radiochemical techniques. The results are expressed as the mean ± SEM with four animals for each observation. △—△ control; ▲—▲ 50 μM; ○—○ 100 μM; ■—■ 200 μM putrescine.

rate of uptake being maintained throughout (Fig. 4b). Thus there appeared to be a maximal inhibitory effect of putrescine that resulted in only a partial inhibition of [$^{14}$C]cystamine uptake.

To characterise the two kinetically defined components of cystamine uptake, studies with various metabolic inhibitors were carried out at either 5 or 100 μM [$^{14}$C]cystamine, whose uptake would be mediated predominantly by the high and low affinity processes respectively. At 4°, the accumulation of 5 μM [$^{14}$C]cystamine was decreased from 58.8 ± 4.3 to 8.0 ± 0.4 nmol/g wet weight/hr, and of 100 μM from 207.7 ± 17.1 to 89.0 ± 10.0 nmol/g wet weight/hr. Uptake at 5 μM was decreased from 82.1 ± 2.9 to 64.9 ± 6.1 nmol/g wet weight/hr in the presence of 1 mM KCN, whilst at 100 μM, the same treatment increased uptake from 183.7 ± 1.0 to 247.0 ± 26.5 nmol/g wet weight/hr (Table 1).

*Pulmonary metabolism of cystamine*

Thin layer chromatography of the acid-soluble cellular material subsequent to incubation with [$^{14}$C]cystamine resulted in the separation of [$^{14}$C]activity into three fractions. Two of these co-chromatographed in both solvent systems (see Methods) with standards of cystamine and taurine (2-aminoethanesulphonic acid), whilst the other has not yet been identified. Some radioactivity remained associated with the acid-precipitated material in a manner that was stable to both organic and inorganic solvents, and may therefore represent a covalent association. (N.B. The incubation of [$^{14}$C]cystamine in the absence of lung slices gave rise to a single peak subsequent to TLC, which co-chromatographed with a cystamine standard. The formation of the two other acid-soluble peaks was therefore shown to be dependent on the presence of lung tissue (results not shown).)

The accumulation of [$^{14}$C]taurine derived from [$^{14}$C]cystamine in the slice was dependent on the cystamine concentration of the medium. Incubation with 5 μM and 100 μM [$^{14}$C]cystamine resulted in approximately 80% and 25% respectively of the cellular radiolabel co-chromatographing with

Table 1. Characteristics of cystamine and putrescine uptake by lung slices

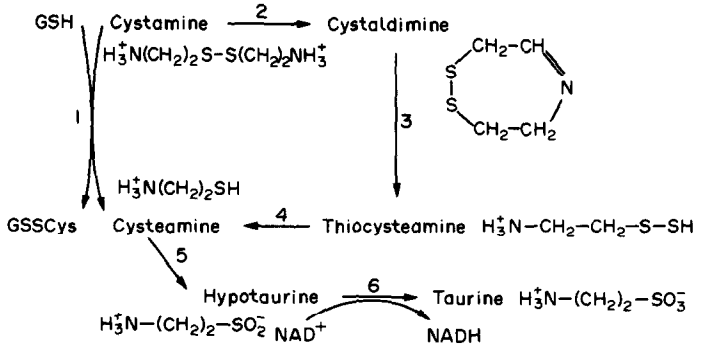
| Compound | $K_m$ (μM) | $V_{max}$ (nmol/g/hr) | Inhibition at 4° % of control | Inhibition by KCN % of control | Na-Free medium % of control | Putrescine inhibition |
|---|---|---|---|---|---|---|
| Putrescine | 11 | 635 | 10*[1] | 5*[1] | 114[2] | |
| Cystamine | 12 | 530 | 14* | 79* | 102 | + |
| | 503 | 5900 | 43* | 134 | | − |

Slices of rat lung were incubated in KRP glucose medium containing [$^{14}$C]putrescine or [$^{14}$C]cystamine under the conditions indicated. The accumulation of $^{14}$C-label was measured using radiochemical techniques. The results are expressed as the means of four observations.

[1] Gordonsmith RH [12].

[2] Smith LL and Wyatt I [7].

* $P < 0.05$.




1. Postulated thiol-disulphide exchange;
2. Amine:oxygen oxidoreductase (deaminating) (EC 1.4.3.6);
3. Spontaneous; 4. uncharacterised;
5. Cysteamine dioxygenase (EC 1.13.11.19);
6. Hypotaurine dehydrogenase (EC 1.8.1.3).

Fig. 5.

[$^{14}$C]taurine (Table 2). The occurrence of the second radiolabeled compound (M2) in the slice was associated only with the uptake of 100 $\mu$M [$^{14}$C]cystamine, and was not detected at the lower [$^{14}$C]cystamine concentration.

The measured cellular levels of [$^{14}$C]taurine, [$^{14}$C]M2 and [$^{14}$C]protein were all decreased by 80% or greater following incubation in either 5 or 100 $\mu$M [$^{14}$C]cystamine at 4°. [$^{14}$C]Cystamine levels were also decreased, although by only 57% at 5 $\mu$M external concentration and by 19% at 100 $\mu$M, the latter not achieving statistical significance (Table 2).

Since even at high cystamine concentrations, high affinity uptake would contribute to some extent to total uptake, to accurately study the low affinity system necessitated the inhibition of this process. This was achieved by incubating the slices in medium containing 100 $\mu$M [$^{14}$C]cystamine, and an excess (10 mM) of putrescine, to abolish high affinity uptake. This resulted in a small, and statistically insignificant decrease in cellular [$^{14}$C]cystamine and [$^{14}$C]taurine, but significant decreases in the levels of [$^{14}$C]M2 and [$^{14}$C]protein.

*Route of metabolism of* 5 $\mu$M [$^{14}$C]*cystamine*

The amine oxidase inhibitors KCN (1 mM) and aminoguanidine (1 mM) did not significantly decrease the levels of radioactivity associated with [$^{14}$C]cystamine, [$^{14}$C]taurine, [$^{14}$C]M2 or with cellular protein (Table 3).

Incubation of lung slices with diethyl maleate (DEM; 1 mM), resulted in a decrease in cellular NPSH levels from 2.20 ± 0.04 to 0.51 ± 0.09 nmol/g wet weight, and an associated decrease in cellular [$^{14}$C]taurine (Table 3). [$^{14}$C]Cystamine levels were slightly, though not significantly, elevated. Protein-associated label was increased by a factor of 2 by DEM treatment.

Dihydroxyethyldisulphide (DHED; 500 $\mu$M) caused a significant decrease in cellular [$^{14}$C]taurine, but, unlike DEM, did not significantly increase protein-associated radioactivity. Cellular [$^{14}$C]cysta-

Table 2. State of radioactivity in lung slice and medium subsequent to 30 min incubation in [$^{14}$C]cystamine

| | | | nmol/ml (Medium) or g wet wt (Slice) | | | |
|---|---|---|---|---|---|---|
| [Cystamine] | Treatment | | M2 | Taurine | Cystamine | Protein-bound |
| 5 $\mu$M | 37° | Medium | N.D. | N.D. | 3.1 ± 0.2 | N.D. |
| | | Slice | N.D. | 54.7 ± 1.4 | 9.2 ± 0.8 | 4.7 ± 0.2 |
| 5 $\mu$M | 4° | Medium | N.D. | N.D. | 3.7 ± 0.0 | N.D. |
| | | Slice | N.D. | 0.3 ± 0.1 | 4.0 ± 0.1 | N.D. |
| 100 $\mu$M | 37° | Medium | N.D. | N.D. | 77.8 ± 1.7 | N.D. |
| | | Slice | 29.1 ± 3.4 | 45.3 ± 4.4 | 72.4 ± 6.3 | 23.9 ± 3.8 |
| 100 $\mu$M | 4° | Medium | N.D. | N.D. | 84.3 ± 1.2 | N.D. |
| | | Slice | 6.3 ± 1.6 | 6.3 ± 1.7 | 59.0 ± 8.8 | 4.0 ± 0.4 |
| 100 $\mu$M | 10 mM | Medium | N.D. | N.D. | 79.2 ± 1.5 | N.D. |
| | Putrescine | Slice | 13.7 ± 0.8 | 41.7 ± 4.3 | 63.7 ± 8.2 | 17.4 ± 3.5 |

Lung slices were incubated in KRP glucose medium containing [$^{14}$C]cystamine at the above concentrations and under the indicated conditions. The $^{14}$C-labeled compounds present were separated by thin layer chromatography and measured by radiochemical techniques. The results are expressed as the mean ± SEM with four animals for each observation. (N.D.—none detected).

Table 3. State of 5 μM [$^{14}$C]cystamine derived radiolabel in lung slice after various treatments

| | % of Control | | | |
|---|---|---|---|---|
| | [$^{14}$C]M2 | [$^{14}$C]Taurine | [$^{14}$C]Cystamine | [$^{14}$C]Protein |
| KCN (1 mM) | N.D. | 102 | 127 | 112* |
| AG (1 mM) | N.D. | 87 | 92 | 92 |
| DEM (1 mM) | N.D. | 52* | 113 | 210* |
| DHED (500 μM) | N.D. | 14* | 116 | 93 |

Lung slices were incubated in KRP glucose medium at 37° containing 5 μM [$^{14}$C]cystamine in the presence or absence of the indicated compounds. The $^{14}$C-labeled compounds present after 30 min incubation were separated by TLC and measured using radiochemical techniques. The results are expressed as the mean with four animals for each observation. (N.D.—none detected).

* $P < 0.05$.

mine levels also remained unaltered by this treatment (Table 3).

## DISCUSSION

Although it had originally been observed that the aminothiol cysteamine is able to inhibit the uptake of paraquat into lung slices [11], our subsequent studies have shown that the cysteamine SH group is unstable in KRP, and that the maintenance of the SH group by DTT abolishes the inhibitory effect of cysteamine. It appears probable, therefore, that as with other thiol compounds [13], cysteamine undergoes autooxidation in medium containing bivalent metal ions, and that its disulphide, cystamine, is responsible for the observed inhibition of paraquat uptake. Indeed, this is consistent with the observation that at equimolar concentrations, cystamine is a more potent inhibitor of paraquat uptake than cysteamine (since the oxidation of two molecules of cysteamine is required to yield one of cystamine).

Furthermore, as cystamine more closely adheres to the structural requirements for transport through the polyamine uptake system [12], it was suggested that the disulphide rather than the thiol may act as a substrate for the accumulation process.

### *Cystamine accumulation*

Several observations suggest that cystamine is in fact transported into lung slices by two separate processes, one of which is that also used in the uptake of the diamine, putrescine.

(i) The measured rate of [$^{14}$C]cystamine uptake describes a biphasic relationship to the cystamine concentration, both components of which obey saturation kinetics (Fig. 3).

(ii) The apparent kinetic parameters of putrescine and high affinity cystamine uptake show a marked similarity, and differ significantly from those of the low affinity process (Table 1).

(iii) The uptake of both putrescine and low concentrations of cystamine is inhibited by KCN and by 4° (Table 1), indicating that both represent active accumulatory processes, and are not the result of simple diffusion, or non-specific ionic binding between the dication substrate and negatively charged cellular structures such as lipids, polynucleotides etc. Furthermore, the uptake of both compounds is seen to be $Na^+$-independent (Table 1).
The uptake of cystamine at high concentrations show different kinetic parameters to that at low concentrations or to that of putrescine, and furthermore is not inhibited by KCN or putrescine.

(iv) The uptake of [$^{14}$C]putrescine is competitively inhibited by cystamine in a manner that is directly related to the cystamine concentration (Fig. 4a). This implies that the uptake of putrescine occurs through a unique transport system, for which cystamine competes. However, although the uptake of cystamine is competitively inhibited by 50 μM putrescine, further increases in the putrescine concentration have little further inhibitory effect (Fig. 4b). We have concluded, therefore, that there are two components of cystamine uptake; one that can be competitively inhibited by putrescine and one that can not.

Although its high capacity suggests that the low affinity system may contribute to cystamine uptake by the lung *in vivo*, the observed kinetics suggest that at the proposed endogenous concentration of cystamine (1 μM) [15], 80% of total uptake would occur through the high affinity system. However, it is possible that the two processes may occur in separate cell types, in which case the role of the low affinity system may be biologically significant. Although the cellular compartments into which cystamine is accumulated have not been established, an analogy may be made with putrescine, which also utilises the high affinity system, and is accumulated by alveolar epithelial type I and type II cells, as well as by Clara cells [7].

### *Pulmonary metabolism of cystamine*

The metabolism of cystamine to taurine has been investigated in several studies [16, 17]. It is now generally accepted that the process initially involves the conversion of the disulphide to the thiol, cysteamine, which is then oxidised through hypotaurine to taurine [18, 19]. Although enzymes catalysing the oxidation of cysteamine and hypotaurine have been identified [19–21], the production of cysteamine from cystamine is less clear. In hog kidney, cystamine is

oxidatively deaminated by a diamine oxidase (EC 1.4.3.6) to form a Schiff base, cystaldimine, which is then broken down to thiocysteamine and subsequently to cysteamine [22, 23]. However, in the liver, cysteamine may be derived by either chemical or enzyme-mediated thiol-disulphide exchange between cystamine and GSH [24].

The data presented suggest that, subsequent to its accumulation by the lung slice, cystamine has several metabolic fates. Taurine appeared as the major metabolite after uptake by both high and low affinity systems, the second metabolite, M2, being found only in association with low affinity uptake. The protein-associated radioactivity probably represents a protein–cysteamine mixed disulphide (cystamine has been identified as a substrate of a protein-thiol transferase [24], although the occurrence of this enzyme in the lung has not been established). Significant intracellular levels of the parent compound, [$^{14}$C]cystamine were also found subsequent to uptake by both systems. However, physiologically, cystamine exists in an equilibrium with cysteamine [13] and since an accurate method is not available for the separation of the two, what we have termed the "cystamine" fraction is in fact a measurement of total [$^{14}$C]cystamine plus [$^{14}$C]cysteamine.

After uptake, although [$^{14}$C]cystamine (5 $\mu$M) is readily metabolised, free [$^{14}$C]cystamine nevertheless accumulates to a concentration in excess of that in the medium. Moreover, at 4° this process is abolished, and the intracellular [$^{14}$C]cystamine concentration reaches only 80% of that in the medium (Table 2). Since the water diffusion space of the lung slice is approximately 0.8 of the total volume [25], these observations infer that 5 $\mu$M cystamine is accumulated by an active process, which is abolished at 4° to allow uptake only by diffusion.

The intracellular free [$^{14}$C]cystamine concentration subsequent to 100 $\mu$M uptake was approximately equal to that in the medium, whilst that at 4° again approximated to 80% of the exogenous concentration, indicating that at this concentration, only a small proportion of the intracellular free [$^{14}$C]cystamine is derived by an active process.

To investigate the role of high affinity uptake at this concentration, an excess (10 mM) of putrescine was added to the incubation medium such that this process would effectively be abolished. The resulting intracellular [$^{14}$C]cystamine concentration of 0.75 of that in the medium indicates that in the absence of the high affinity system, cystamine itself is not accumulated as such, but is able to freely diffuse into the lung slice. We have concluded, therefore, that the low affinity uptake system represents the diffusion of cystamine into the cell and its subsequent metabolism, and that the saturable site associated with the process is intracellular rather than associated with the plasma membrane.

We have observed that in lung slices the production of [$^{14}$C]taurine from 5 $\mu$M [$^{14}$C]cystamine is not significantly altered in the presence of the DAO inhibitors aminoguanidine or KCN (Table 3), and therefore suggest that this enzyme is not important in the pulmonary metabolism of cystamine. However, treatment with dihydroxyethyldisulphide (DHED), an analogue of cystamine in which the amino groups are substituted by hydroxyl groups, resulted in a marked inhibition of [$^{14}$C]taurine production, suggesting that in the lung, cystamine is metabolised at the disulphide group rather than at the amino group.

Furthermore, the GSH depleting agent, DEM, was observed to significantly decrease both NPSH levels and the production of [$^{14}$C]taurine from 5 $\mu$M [$^{14}$C]cystamine in the lung slice, indicating that the metabolism of 5 $\mu$M [$^{14}$C]cystamine is GSH-dependent. It is possible, therefore, that the initial step in the pulmonary metabolism of 5 $\mu$M [$^{14}$C]cystamine involves a thiol-disulphide exchange between cystamine and GSH to yield a glutathione–cysteamine mixed disulphide and free cysteamine (Fig. 5). The relative cellular levels of [$^{14}$C]cystamine and its metabolites after incubation in 5 and 100 $\mu$M [$^{14}$C]cystamine suggest that at the postulated endogenous concentration of 1 $\mu$M, the disulphide is extensively metabolised by the lung to taurine, although low intracellular concentrations of free cystamine and its protein-bound derivative may also be present. As the [$^{14}$C]taurine formed was wholly retained by the lung slice (Table 2), we have concluded that the pulmonary uptake of cystamine differs from other uptake systems by which the lung is known to regulate plasma levels of several vasoactive amines, such as 5-hydroxytryptamine, through a process of accumulation, oxidative deamination and release [26]. Moreover, these results raise the possibility of an endogenous function for taurine in the lung.

Taurine is found in high concentrations in several tissues that generate high levels of reactive oxygen species [27], and amongst its other effects, has been shown to possess antioxidant properties [27–30]. Moreover, several disulphides, including cystamine have been suggested to have the potential, through protein-*S*-thiolation of specific enzymes, to regulate cellular NADPH levels in response to oxidative stress [15]. Since the apparent $K_m$ for the pulmonary uptake of cystamine (12 $\mu$M) is similar to the plasma concentration of the disulphide (1 $\mu$M), it is likely that *in vivo*, this system mediates the accumulation into the lung of cystamine and consequently taurine, both of which may subsequently be used as antioxidants.

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