The Reduction of Cystine During its Transport into Rat Jejunal Everted Segments and Sacs

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1. Everted segments and sacs of rat jejunum were incubated in buffer containing [^{35} S]cystine. 2. Concentration gradients were achieved by both segments and sacs, and the effects of duration of incubation and of cystine concentration on the isotope distribution ratios were determined. 3. Kinetic constants were determined for the uptake of cystine by both segments and sacs, and the differences between the two systems are discussed. 4. Reduction to cysteine was virtually complete intracellularly and in the sac lumen. Extensive reduction in the medium occurred only when segments were incubated. 5. Anaerobiosis prevented a concentration gradient being obtained between the medium and the tissue, but had little effect on the extent of reduction to cysteine in the tissue and sac lumen. 6. It is concluded that cystine is transported by an active process into rat jejunum, where it is present almost entirely in the reduced form, and that efflux of cysteine occurs through the serosal surface.

The use of the everted gut sac in studies of transport of organic solutes was first described by Wilson & Wiseman (1954). Neil (1959) compared the transport of cystine and cysteine in the everted sac preparation with the transport of these amino acids during luminal perfusion of the small intestine in an intact rat and showed that a concentration gradient could be obtained for cystine between the mucosal and serosal surfaces of the sac preparation. An alternative technique *in vitro* is the use of everted gut segments and it has been found that the two complementary techniques do not always yield the same information (cf. McLeod & Tyor, 1967).

Preliminary studies on the transport of cystine in rat everted gut segments have been carried out by Crawhall & Segal (1967), who showed that the cystine transported into the gut segments was present in the tissue in the reduced state as cysteine. In addition it was found that 36% of the cystine in the incubation medium was reduced to cysteine after 30 min. This could have occurred either as the result of direct reduction in the medium by tissue enzymes or as a result of efflux of cysteine from the tissue. If the former had occurred then the molecule being transported would have been cysteine and not cystine. Previous studies of cystine transport in gut mucosa obtained by peroral biopsy of patients with cystinuria indicated that no reduction took place before

transport (Rosenberg, Crawhall & Segal, 1967). These authors showed that although mechanisms for active transport of cystine were missing in jejunal mucosal biopsy tissue from patients with cystinuria, transport of cysteine still proceeded at the normal rate. If reduction of cystine to cysteine had occurred before transport there should have been no deficit of cystine transport in these patients. It was also shown that intracellular reduction of cystine in these patients was similar to that in normal controls. The other evidence for separate transport mechanisms was derived from kinetic studies of cystine and cysteine transport in the rat kidney cortex *in vitro* (Segal & Crawhall, 1968) and in human kidney cortex *in vitro* (Segal & Crawhall, 1967). We have now investigated in more detail the kinetics of cystine transport in rat jejunal everted segments and the conditions under which reduction occurs.

The everted gut segment has two different transporting surfaces, the mucosal and serosal surfaces. In the segment the transport characteristics are a combination of the characteristics of the two surfaces. In the everted sac the characteristics of the mucosal surface can be separated from the serosal surface. In this system the serosal surface is not in contact with the main portion of the incubation medium, so that efflux of the amino acid via the serosal surface would become isolated within the sac. Analysis of the sac contents would give a direct measure of the degree of reduction that the

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cystine had undergone while passing through the tissue, and the tendency to aerobic reoxidation of cystine would be decreased because the contents of the sac are isolated from the gaseous phase during the incubation.

MATERIALS AND METHODS

Chemicals. L-[^{35}S]Cystine was obtained from Schwarz BioResearch Inc. (Orangeburg, N.Y., U.S.A.), and unlabelled L-cystine from Hopkin and Williams Ltd. (Chadwell Heath, Essex). The required concentration of radioactive cystine in the incubation medium was obtained by the addition of appropriate amounts from a 2 mM solution of the [^{35}S]cystine and a 38 mM solution of the unlabelled cystine, both made up in dilute NaOH solution because of the low solubility of cystine in water. NEM† was obtained from Calbiochem (Los Angeles, Calif., U.S.A.).

Preparation of tissue. Male Sprague-Dawley rats (aged 6 weeks, and weighing approx. 150 g.) were fed on Rat Chow diet (Dixon's Feeds 41B, Crane Mead Mills, Ware, Herts.) and starved for 24 hr. before use. The rats were stunned and decapitated, and the upper small intestine was removed from each. The section between 5 and 15 cm. from the pylorus was everted and either cut into segments 2–3 mm. long (Marsh, Rosenberg & Segal, 1962) or else made into two sacs by the method of Wilson & Wiseman (1954). The sacs were weighed empty and then filled with Krebs-Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), and weighed again. The average volume of buffer required to fill the sacs was 0.25 ml.

Incubation procedure. Three single segments from three separate rats were incubated, in 25 ml. conical flasks at 37°, in 2 ml. of Krebs-Ringer bicarbonate buffer containing the appropriate concentration of [^{35}S]cystine. The flasks were gassed for 30 sec. with $\text{O}_2 + \text{CO}_2$ (95:5) and sealed with rubber turnover closures (Suba-Seal Works, Barnsley, Yorks.) and the flasks were shaken at 37° for the determined period of time in a metabolic shaker (A. Gallenkamp and Co. Ltd.). At the completion of the incubation the tissue was removed, rinsed twice in 0.9% NaCl solution, blotted and weighed on a torsion balance (Roller Smith Precision Balances, Federal Pacific Electric Co., Jersey City, N.J., U.S.A.).

Sacs were incubated separately in a similar manner in 100 ml. conical flasks containing 20 ml. of Krebs-Ringer bicarbonate buffer. After being rinsed, blotted and weighed, the sacs were opened and the contents allowed to drain into a centrifuge tube (10 ml.) containing NEM (0.5 ml., 20 mM in 0.1 M-sodium phosphate buffer, pH 7.4) and the sacs then reweighed. By this means it was possible to ensure that the sacs had been correctly sealed and any change in fluid volume was determined. The subsequent procedure was varied, depending on whether isotope distribution ratios or cystine/cysteine ratios were being determined.

Determination of isotope distribution ratios. After incubation, the three segments were placed in 2 ml. of water and the single sacs in 4 ml. of water, both contained in centrifuge tubes (10 ml.). These were heated in a water bath at 100° for 6 min. Portions (0.2 ml.) of the tissue extract, the fluid from inside the sac and the incubation medium (diluted

1:10 with water) were taken for radioactivity determination. To each 0.2 ml. portion of aqueous solution, 2.8 ml. of ethanol and 7.0 ml. of toluene-based phosphor [2-(4-*tert*-butylphenyl)-5-(4-biphenyl)-1-oxa-3,4-diazole; Ciba, Duxford, Cambridge] were added. Radioactivity was determined in a Nuclear-Chicago automatic liquid-scintillation counter. The calculation of the isotope distribution ratios was carried out by the method described by Rosenberg, Blair & Segal (1961), by using the information that the total tissue water of rat jejunum was 84% of the tissue weight and the extracellular space as determined by [^{14}C]inulin uptake was 12% of the tissue weight (Crawhall & Segal, 1967).

Determination of the extent of cystine reduction. At the completion of the incubation a portion of the medium (0.2 ml.) was added to NEM (1.8 ml., 20 mM in 0.1 M-sodium phosphate buffer, pH 7.4). The NEM blocks the free thiol group of cystine and thus prevents reoxidation to cystine (Smyth, Nagamatsu & Fruton, 1960). The segments and sac tissue were also placed in NEM solution (1.0 ml. and 2.0 ml. respectively) and homogenized in a glass tissue grinder (Kontes Glass Co., Vineland, N.J., U.S.A.). After 1 min. trichloroacetic acid (10%, 1.0 ml. for segments and 2.0 ml. for sacs) was added to both these NEM solutions. The sac contents were also added to NEM solution (0.5 ml.) and after 1 min. trichloroacetic acid (10%, 0.5 ml.) was added. Protein precipitated by the trichloroacetic acid was removed by centrifugation, and the trichloroacetic acid subsequently removed by ether extraction (3×1 ml.).

Portions (10 μl .) of the various remaining solutions were submitted to high-voltage electrophoresis (model 2, Miles Hivolt, Shoreham, Sussex) in Whatman 3MM paper, and 6.8% (v/v) formic acid, at 4000 v for 30 min. between two plates cooled by running water at room temperature. Marker control spots of cystine and Cys-NEM were applied to the electrophoresis paper alongside the radioactive samples. At the completion of the electrophoresis, the paper was dried, and the marker lanes were cut out and stained with ninhydrin-cadmium acetate reagent (Crawhall & Segal, 1967) to show the approximate positions of the radioactive bands. The lanes to which the radioactive solutions had been applied were cut out and divided into 2 cm. sections. These were immersed in 10 ml. of the toluene-based phosphor in liquid-scintillation vials, and the extent of reduction of cystine was calculated from the radioactivity observed in the areas corresponding to cystine and Cys-NEM.

RESULTS

Uptake of cystine by segments. The uptake of [^{35}S]cystine was studied at increasing concentrations of cystine under aerobic conditions (Fig. 1). The results are expressed as isotope distribution ratios. At the two low concentrations, 0.025 and 0.2 mM, similar ratios were observed for incubation periods up to 30 min., indicating that no saturation of the transport mechanism was occurring. The isotope ratios obtained at 0.95 mM concentration were not so great, indicating some saturation of the transport mechanism. Under anaerobic conditions at 0.025 mM, an isotope distribution ratio of only

† Abbreviations: NEM, *N*-ethylmaleimide; Cys-NEM, 2-(L-2-amino-2-carboxyethylthio)-*N*-ethylsuccinimide.

Fig. 1. Effect of incubation time on the uptake of [^{35}S]-cystine (expressed as isotope distribution ratio) by everted gut segments for various concentrations ($\circ$, 0.025 mM; $\square$, 0.2 mM; $\triangle$, 0.95 mM) and under anaerobiosis ($\bullet$, 0.025 mM).

Fig. 2. Effect of concentration of [^{35}S]cystine in the incubation medium on its uptake (expressed as isotope distribution ratio) by everted gut segments for various incubation times ($\circ$, 10 min.; $\square$, 15 min.; $\triangle$, 20 min.; $\bullet$, 30 min.) and under anaerobiosis ($\blacksquare$, 15 min.).

Fig. 3. Effect of diffusion on the uptake of [^{35}S]cystine (expressed as isotope distribution ratio) by everted gut segments, as revealed by plotting the reciprocal of the concentration of cystine in the incubation medium for different incubation times ($\circ$, 10 min.; $\triangle$, 20 min.).

1.5 was achieved after 60 min. of incubation. The effect of concentration of substrate on the distribution ratio for different times of incubation is shown in Fig. 2. For all periods of incubation up to 30 min. the achievement of a concentration gradient was proceeding faster at the lower concentrations than at 0.95 mM. Extrapolation of these lines would soon reach a point where no gradient was achieved in 30 min., but this cannot be determined experimentally because of the limitation of the solubility of cystine. The inhibiting effect of anaerobiosis (nitrogen + carbon dioxide, 95:5) on cystine transport over the whole range of concentration was demonstrated, although at a concentration of 1 mM equilibration between the intracellular and extracellular isotope concentrations had not been achieved in 15 min. The graph of isotope distribution ratio plotted against the reciprocal of the concentration of cystine in the medium has been used to demonstrate that at high concentrations diffusion of substrate becomes more significant than mediated transport and hence that a limiting isotope distribution ratio of 1 is obtained (Akedo & Christensen, 1962). The data shown in Fig. 3 are compatible with this result, but the extrapolation of these lines is not as reliable with cystine because concentrations of substrate greater than 1 mM cannot be obtained.

In Fig. 4 the influx of cystine has been calculated from the linear part of the uptake curves for various concentrations of cystine and plotted against the concentration of cystine. The ratio of uptake increases with increasing concentration of cystine, but the increase of rate begins to fall off above 0.4 mM and then continues to increase at a linear rate up to 0.95 mM. The ratio of uptake at various concentrations of cystine under anaerobic conditions is shown on the lower curve (Fig. 4). This proceeds at a lower rate and is directly proportional to cystine concentration. The rate of increase of uptake of cystine under anaerobic conditions is similar to that under aerobic conditions at the higher cystine concentrations (i.e. the two graphs become parallel). This could be explained if the transport mechanism was approaching saturation at the higher concentrations under aerobic conditions and that cystine was then entering the tissue principally by non-mediated transport in both the aerobic and anaerobic systems.

In Fig. 5 the rate of transport of cystine observed in a series of separate experiments is plotted as a double-reciprocal graph of rate against concentration (Lineweaver & Burk, 1934). The values appear to correspond to a straight line and hence there is no evidence for more than one transport system on the basis of differing K_m values. The rate of uptake of cystine was also investigated in the presence of a fixed concentration of lysine. The results are also

Fig. 4. Effect of concentration of [^{35}S]cystine in the incubation medium on its uptake (expressed as transport rate) by everted gut segments during aerobic incubation ($\circ$, mean values for different incubation times used, since the rate remains constant for up to 60 min. incubation for a given concentration of cystine) and during anaerobic incubation ($\bullet$, 15 min. incubation).

Fig. 5. Effect of lysine on the rate of transport of [^{35}S]cystine by everted segments, shown by plotting the reciprocal of the rate of transport of cystine alone ($\circ$) and of cystine in the presence of 2 mM-lysine ($\bullet$) against the reciprocal of cystine concentration.

plotted in a double-reciprocal graph in Fig. 5; the intercept of that straight line with the ordinate coincides with that for cystine, indicating that $V_{\max}$ is unchanged but that the K_m is increased. These results are compatible with lysine being a competitive inhibitor of cystine transport. No correction has been made for the diffusion component of cystine transport, partly because of the uncertainty of this value as determined from Fig. 3 and partly because the rates of cystine transport become so low in the presence of lysine inhibition that no meaningful results would be obtained if the diffusion factor were subtracted. The observed K_m was $0.67 \times 10^{-3} \text{ M}$ and $V_{\max}$ $0.11 \mu\text{mole/g. wet wt. of tissue/min.}$

Reduction of cystine in segments. The extent of intracellular reduction of cystine after incubation

Table 1. *Effect of cystine concentration and anaerobiosis on the extent of reduction by everted gut segments*

Tissue was incubated in buffer containing [^{35}S]cystine at 37° for 15 min.; the products were treated with NEM solution and separated by high-voltage electrophoresis.

Concn. of cystine in medium (mM)	Reduction to cystine (%)			Concn. of cystine in medium (mM) (aerobic)
	In tissue		In medium (aerobic)	
	Anaerobic	Aerobic		
0.025	74	87	22	0.011
0.05		98	30	0.029
0.10		92	17	0.040
0.20	70	93	13	0.055
0.95	48	83	7	0.141

Table 2. *Effect of incubation time on the extent of reduction of cystine to cystine in the medium by everted gut segments*

Tissue was incubated for different times in buffer containing [^{35}S]cystine at both low and high concentrations (0.025 mM and 0.95 mM) at 37° . The medium was then treated with NEM solution and the products were separated by high-voltage electrophoresis. Results are expressed as

$$\% \text{ cystine} = \frac{\text{counts as cystine} \times 100}{\text{total counts (cystine + cysteine)}}$$

Incubation time (min.)	Cystine in medium (%)	
	0.025 mM-Cystine	0.95 mM-Cystine
10	15	5
15	22	7
20	—	10
30	28	10

in increasing concentrations of cystine in the medium is shown in Table 1. Under anaerobic conditions (nitrogen + carbon dioxide, 95:5) the reduction of cystine in the tissue appears to be less than under aerobic conditions. This may be partly explained on the basis of reduction being an energy-requiring process dependent on the availability of oxygen and it may be partly explained by the intracellular concentration of cystine being less under anaerobic conditions and the final result being more affected by cystine from the medium being trapped in the extracellular fluid. Determinations of the extent of reduction have not been corrected for cystine trapped in the extracellular fluid. Some reduction of cystine occurs in the medium but proportionately this decreases rapidly as the cystine concentration in the medium is increased. If the results of the reduction in the medium are expressed as total amount of cystine

Fig. 6. Effect of incubation time on the uptake by everted gut sacs of [^{35}S]cystine (0.2 mM), expressed as rate of uptake by tissue from medium ($\circ$), efflux from tissue into sac contents ($\square$), and the corrected overall rate of transport by the tissue (Δ).

formed from cystine, it can be seen that much more cystine is formed at higher concentrations of cystine and this increases with increasing time of incubation (Table 2). The percentage reduction of cystine in the tissue remained high and unchanged between 10 and 30 min. incubation.

Uptake of cystine by sacs. Everted gut sacs containing buffer without any added cystine were incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) containing [^{35}S]cystine (0.2 mM) for different times varying from 15 to 180 min. (Fig. 6). The rate of uptake of amino acid by the tissue can be calculated from the radioactivity contained within the tissue. Fig. 6 shows that the apparent influx falls quite rapidly with time if influx is calculated directly from the tissue content of radioactivity. The radioactivity within the sac lumen must have passed through the tissue before reaching the lumen, and so in calculating the total tissue influx the amount transported on into the lumen can be added to that found to be present in the tissue. This has been done in the upper line of Fig. 6, which shows that the initial influx is approx. 10 nmoles/g. of tissue wet wt./min., and this apparently falls off at the rate of 2 nmoles/g. of tissue wet wt./min./hr. The initial influx can be calculated fairly accurately by extrapolating this graph back to time zero, and the decrease of influx may be the result of various factors such as loss of viability of the tissue during 3 hr. incubation or efflux of radioactive substrate back into the medium, either from the tissue or from the sac lumen via the tissue.

In Fig. 7 these results are expressed as isotope distribution ratios, showing that at 60 min. equilibrium is reached between the influx of substrate into the tissue and efflux of substrate both into the medium and the sac lumen. The appearance of cystine inside the sac is shown on the other curves

Fig. 7. Effect of incubation time on the uptake by everted gut sacs of [^{35}S]cystine (0.2 mM), expressed as the isotope distribution ratio between tissue and medium ($\circ$), between sac contents and tissue ($\square$) and between sac contents and medium (Δ).

in Fig. 7. The achievement of a gradient between the sac and the medium is dependent first on the uptake of cystine by the tissue and then on the efflux of this from the tissue into the sac, and hence is slower than the achievement of a gradient in the tissue itself. It is not possible to be sure from these data whether efflux from the tissue into the sac is mediated transport or simple diffusion because no gradient of cystine concentration is achieved between the solution within the sac and the tissue from which it has been derived. Also, as shown in Fig. 8 the rate of transfer of radioactive cystine from the tissue into the sac lumen is directly proportional to the cystine concentration in the tissue, unlike the influx of cystine from the medium into the tissue which shows a decrease of rate of transport at higher concentrations of cystine in the medium. It is possible that cystine transport from the tissue into the sac lumen might have different kinetic properties, which would not show this saturation effect, but at present there is no evidence for mediated transport from the serosal surface.

The rate of transport was also studied at different concentrations for a fixed period of time (30 min. incubation). Fig. 8 shows that the rate of accumulation of radioactivity in the tissue is non-linear at higher concentration of cystine in the medium. To obtain a true measure of the rate of cystine transport the amounts of cystine present in the tissue and the lumen have been added together and the upper curve in Fig. 8, which is based on this calculation, shows that the rate of uptake becomes almost linear with respect to cystine concentration in the medium except at the highest concentration.

The ability of the tissue to maintain a concentration gradient at various concentrations of cystine in the incubation medium is shown in Fig. 9. At low concentrations of cystine (0.025 mM) an average tissue/medium ratio of 3.1 was achieved, but at increasing concentrations the ability of the tissue

Fig. 8. Effect of concentration of $[^{35}\text{S}]$ cystine in the incubation medium on its uptake by everted gut sacs (30 min. incubation), expressed as rate of uptake by tissue from medium (○), efflux from tissue into sac contents (□) and corrected overall rate of transport by the tissue (Δ).

Fig. 9. Effect of concentration of $[^{35}\text{S}]$ cystine in the incubation medium on its uptake by everted gut sacs (30 min. incubation), expressed as the isotope distribution ratio between tissue and medium (○), between sac contents and tissue (□), and between sac and medium (Δ).

to achieve this concentration gradient was impaired and at 0.95 mM concentration the tissue/medium ratio was close to 1:1. This situation has been attributed to an increasingly important contribution to uptake by the tissue from non-mediated transport or simple diffusion. At higher concentrations of substrate both the observed influx and efflux rates are more dependent on diffusion and hence tissue/medium gradients cannot be achieved. Because of the delay in achieving concentrations in the sacs compared with the tissue, sac/medium and sac/tissue concentration gradients never reach 1 in the incubation period of 30 min. (Wilson & Wiseman, 1954).

A double-reciprocal plot of initial rate of uptake against concentration has been made for the uptake of cystine by the tissue of the gut sac (Fig. 10). If the rate of uptake of isotope into the tissue alone

Fig. 10. Effect of concentration of $[^{35}\text{S}]$ cystine in the incubation medium on its rate of uptake by everted gut sacs (30 min. incubation), as shown by plotting against the reciprocal of cystine concentration the reciprocal of rate of uptake by tissue from medium (○) and the reciprocal of the corrected overall rate of transport by the tissue (Δ).

is considered, a straight line is obtained from which it can be calculated that K_m is $1.1 \times 10^{-3} \text{ M}$ and $V_{\max}$ is $0.056 \mu\text{mol/g. wet wt. of tissue/min.}$ The radioactive amino acid accumulating in the sac is also derived from the amino acid originally present in the medium, so that the rate of accumulation inside the sac should be added to the influx calculated from the tissue alone, giving the overall rate of uptake by the tissue. If this is done a second straight line is obtained (Fig. 10) with a higher $V_{\max}$ of $0.10 \mu\text{mol/g. wet wt. of tissue/min.}$ and K_m $1.7 \times 10^{-3} \text{ M}$. These values have not been corrected for a diffusion factor.

A double-reciprocal plot of the rate of isotope accumulation inside the sac against medium concentration gave a graph that can be considered as passing through the origin, and hence no $V_{\max}$ or K_m could be calculated for this system. It would be desirable to obtain data at higher cystine concentrations so that saturation kinetics could be observed, but this is not possible with cystine because of its low solubility.

Reduction of cystine in the sac lumen. The sac preparation was incubated in Krebs-Ringer bicarbonate buffer containing $[^{35}\text{S}]$ cystine (0.2 mM) at 37° for 30 min. and the tissue was then homogenized with NEM and the radioactive products were separated by high-voltage electrophoresis as described above. Under these conditions it was found (Table 3) that 98% of the radioactivity in the tissue corresponded to the NEM derivative of cysteine. Analysis of the sac contents showed that 97% was present as cysteine, but in the medium only 1% was found to be present as cysteine. In the medium a

Table 3. *Metabolism of cystine by everted gut sacs*

Tissue was incubated in buffer containing [^{35}S]cystine (0.2 mm) at 37° for 30 min. The products were treated with NEM solution and separated by high-voltage electrophoresis.

	Distribution of radioactivity (%)		
	Cystine	Cysteine	Acidic metabolite(s)
Incubation medium	90	1	9
Tissue	2	98	—
Sac contents	2	97	1

Table 4. *Effect of cystine concentration on extent of reduction to cysteine in the sac lumen*

Sacs were incubated for 30 min. in buffer containing [^{35}S]cystine, and the extent of reduction was determined after high-voltage electrophoresis. The numbers of determinations carried out are shown in parentheses.

Concn. of cystine in medium (mM)	Reduction to cysteine (%)	
	Mean	Range
0.025 (6)	96	93–98
0.1 (2)	100	—
0.2 (3)	97	95–99
0.5 (2)	95.5	94–97
0.95 (2)	94.5	93–96

Table 5. *Effect of incubation time on extent of reduction of cystine in sac lumen*

Sacs were incubated for different times in buffer containing [^{35}S]cystine (0.2 mM), and the extent of reduction was determined after high-voltage electrophoresis. The numbers of determinations are shown in parentheses.

Incubation time (min.)	Reduction to cysteine (%)	
	Mean	Range
30 (3)	97	95–99
60 (7)	86	73–97
120 (1)	68	—
180 (2)	55	45–65

small amount of radioactivity was also found after high-voltage electrophoresis in the region corresponding to the acidic metabolites cysteic acid and cysteinesulphonic acid.

Varying the concentration of cystine in the medium made very little difference to the extent of reduction to cysteine in the sac contents (Table 4), which was virtually complete for the whole concentration range investigated. With increasing time of incubation the extent of reduction within

the sac decreased (Table 5), possibly as the result of atmospheric oxidation of the cysteine originally present.

The effect of anaerobiosis on the extent of reduction in the medium and the sac was investigated for the lowest concentration used (0.025 mM, 30 min. incubation) as shown in Table 6. Although anaerobiosis greatly decreased the tissue/medium isotope distribution ratio (a concentration gradient could no longer be obtained) and the rate of cystine uptake by the tissue, the radioactivity that did accumulate inside the sac was still almost entirely in the reduced form. Anaerobiosis did not significantly alter the amount of unreduced cystine remaining in the medium after incubation, but led to an increased extent of reduction in the medium, and decreased the amount of radioactivity corresponding to the acidic metabolites referred to above. This substance did not occur appreciably in the sac contents or the tissue and may be due to oxidation in the medium of cysteine that had effluxed from the tissue.

DISCUSSION

The interpretation of the results of experiments on cystine transport is complicated by the fact that the cystine is reduced to cysteine within the tissue. Previous studies, which are referred to in the introduction, have demonstrated that this reduction takes place after the cystine has entered the tissue. The reduction of cystine to cysteine was confirmed in the experiments described in the present paper, where it was shown that the amino acid leaves the serosal surface of the everted gut sac in the form of cysteine. This process is presumably also taking place in the everted gut segments, but in this case the cysteine returns to the incubation medium. This concept offers a possible explanation for the apparent reduction of some of the cystine to cysteine in the incubation medium when segments were used for the experiments.

The inhibition of cystine transport by lysine was expected in view of some previous studies. Robson & Rose (1957) showed in human subjects that lysine infusion increased the urinary excretion of cystine and the other basic amino acids but in a preparation of rat kidney slices *in vitro* lysine did not inhibit cystine transport (Rosenberg, Downing & Segal, 1962). Cystine has been shown to inhibit lysine transport in the everted segment of rat gut (Segal, Lowenstein & Wallace, 1968) and the experiments described above (Fig. 5) demonstrate the opposite situation where lysine is an inhibitor of cystine transport in this system. The inhibition appears to be competitive although there is doubt about the possibility of correcting the rate of uptake for diffusion into the tissue in highly inhibited systems.

Table 6. *Effect of anaerobiosis on the uptake and reduction of cystine by everted gut sacs*

Sacs were incubated for 30 min. in buffer containing [^{35}S]cystine (0.025 mM) under aerobic and anaerobic conditions. The products were treated with NEM solution and separated by high-voltage electrophoresis. Results are expressed as the means of six determinations, with the experimental ranges shown in parentheses.

Conditions of incubation	Uptake by tissue		Reduction in medium (%)			Reduction in sac (%)	
	Isotope distribution ratio (tissue/medium)	Rate of transport by tissue* (nmoles/g. wet wt. of tissue/min.)					
			Cysteine	Metabolites	Cystine	Cysteine	Metabolites
Aerobic	3.1 (2.7-3.4)	2.6 (2.0-3.1)	11 (6-14)	27 (21-35)	62 (55-66)	96 (93-98)	0 —
Anaerobic	0.42 (0.27-0.54)	0.40 (0.23-0.57)	27 (21-30)	8 (6-12)	65 (58-71)	90 (84-95)	0 —

* Corrected for efflux from tissue into sac lumen.

This fact that cystine is leaving the tissue by the serosal surface can be used to correct kinetic constants obtained from a double-reciprocal plot of the rate of uptake of cystine at different concentrations. The kinetic constants obtained for the transport of cystine in the everted-segments experiments for K_m and V_{max} were $0.67 \times 10^{-3} \text{ M}$ and $0.11 \mu\text{mole/g. wet wt. of tissue/min.}$ respectively. The values found for the sac experiments were $K_m 1.1 \times 10^{-3} \text{ M}$ and $V_{max} 0.056 \mu\text{mole/g. wet wt. of tissue/min.}$ If these are corrected for serosal efflux they become $1.7 \times 10^{-3} \text{ M}$ and $0.10 \mu\text{mole/g. wet wt. of tissue/min.}$ This gives a value of V_{max} closely comparable with that found with segments, but the difference in K_m is probably significant. In the sac experiments there is no component of transport derived from serosal influx, and efflux from the serosal surface has been allowed for. For these reasons it is likely that the results obtained from the sac experiments more accurately represent the characteristics of the mucosal transport systems. It can be seen from the observed K_m value of $1.1 \times 10^{-3} \text{ M}$ that saturation of the transport system for cystine cannot be achieved experimentally because this is close to the limit of solubility of the amino acid at that pH.

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