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# SULPHATE TRANSPORT BY RAT ILEUM
# EFFECT OF MOLYBDATE AND OTHER ANIONS

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## SUMMARY

Kinetic constants for $SO_4^{2-}$ transport by upper and lower rat ileum in vitro have been determined by computer fitting of rate vs concentration data obtained using the everted sac technique. $MoO_4^{2-}$ inhibition of this transport is competitive, and kinetic constants for the inhibition were similarly determined. Transport is also inhibited by the anions $WO_4^{2-}$, $S_2O_3^{2-}$ and $SeO_4^{2-}$, in the order $S_2O_3^{2-} > SeO_4^{2-} \geqq MoO_4^{2-} > WO_4^{2-}$. These anions have no effect on the transport of L-valine. Low $SO_4^{2-}$ transport rates were observed in sacs from animals fed a high-molybdenum diet. The significance of the results with respect to the problem of molybdate toxicity in animals is discussed, and related to the known protective effect of $SO_4^{2-}$.

## INTRODUCTION

$SO_4^{2-}$ transport across the intestine in vitro has been studied in several species of laboratory animal [1–8]. Our interest in this subject arises from the problem of molybdate toxicity in animals, particularly ruminants [9]. The symptoms of molybdosis are, in some circumstances, alleviated by feeding sulphate [10], as well as cysteine and methionine [11] and copper [12, 13]. The syndrome in the rat is characterised by weight loss and anorexia, but ruminants are more susceptible to molybdate and the most striking clinical manifestation is scouring [9]. Reduction by ruminal microflora of ingested $SO_4^{2-}$ [14] occurs quickly and the rate is apparently enhanced by molybdate [15], nevertheless $SO_4^{2-}$ uptake by sheep gut is rapid in vivo [16].

The biochemical basis of these phenomena is not yet established. The main aim of the present work was to study the effect of $MoO_4^{2-}$ both in vivo and in vitro on $SO_4^{2-}$ transport in rat intestine, and to examine the possibility that these anions compete for a common transport system. Such a transport system has been shown for some microorganisms [17]. The authors are unaware of any previous study in mammals of the kinetics of sulphate uptake or the interactions of other anions with the sulphate transport system.

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## METHODS

All experiments were performed on white male Wistar rats weighing 230–250 g unless stated. They were maintained on an unrestricted diet with free access to water.

Everted sacs of small intestine [18] were prepared according to the technique of Barry et al. [19] by dividing the combined jejunum and ileum into five sacs. Sacs IV and V, representing the proximal and distal ileum, were most frequently used. Each sac was filled with 1 ml of bicarbonate saline [20], in which $SO_4^{2-}$ was replaced by $Cl^-$, and incubated with shaking for 30 min at 37 °C in 25 ml of the same medium containing appropriate additions, in equilibrium with a gas phase of $CO_2 : O_2$ (95 : 5, v/v). $SO_4^{2-}$ was added as $Na_2SO_4$ to give the concentrations indicated in the tables, together with trace amounts of carrier free $Na_2{}^{35}SO_4$ (from The Radioachemical Centre, Amersham, England). The other anions shown in the tables were added as the sodium salts.

At the end of the incubation period each sac was washed with saline, the serosal fluid collected, and the gut homogenised and deproteinised using $ZnSO_4$/NaOH. 1-ml samples of each fraction were counted on a Packer Tri-Carb scintillation counter in 10 ml of toluene/Triton-X/PPO/POPOP scintillant [21]. The parameter used for assessing transport was mucosal transfer, taken to be the sum of the amounts accumulated in the serosal fluid and the gut wall. The system of weighings used has been described [22].

In some experiments, sacs were prepared from animals given high levels of $MoO_4^{2-}$ as an 0.5 % solution in drinking water. These animals consumed the normal laboratory diet, but gained weight very slowly.

For experiments on the transport of L-valine, the normal bicarbonate saline, containing sulphate was used. L([$^{14}$C]Valine was added in tracer amounts and the solutions analysed as above.

## RESULTS AND CONCLUSIONS

### *Site of $SO_4^{2-}$ uptake*

The rate of $SO_4^{2-}$ uptake was found to be highest in the lower ileum in vitro; this agrees with previous observations [1, 2, 4, 8]. There is a steady gradient increasing down the intestine (Fig. 1). Subsequent experiments were performed using only sacs IV and V, representing the upper and lower ileum, respectively.

### *Concentration dependence of $SO_4^{2-}$ transport*

There is no previous study of the effect of $SO_4^{2-}$ concentration in the mucosal fluid on the mucosal transfer. Previous studies have often employed very low concentrations [1, 4, 5], and there has been no demonstration that the system is saturable. A concentration range 0.004–7.0 mM was employed, and the sets of data derived for each sac separately (Fig. 2) fitted by regression to the Michaelis-Menten equation using a computed least squares fit [23] (Table I). At least two determinations were performed for most concentrations used, and an analysis of internal and external variance showed that the fit was good. As a check, the data were fitted in three ways, using mucosal transfer values expressed per sac, per cm of intestine measured initially, and per gram of initial wet weight. The $K_t$ values derived by the different methods are

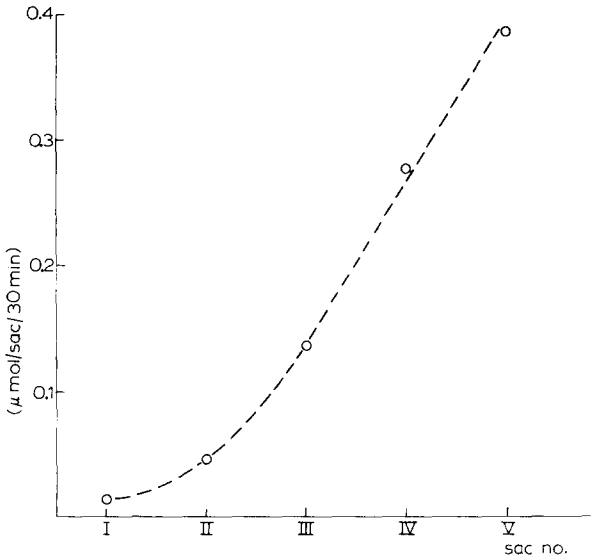


Fig. 1. $SO_4^{2-}$ transport by different parts of rat small intestine. Incubation as described under Methods. Each point is the mean of two determinations. Mucosal transfer is expressed as $\mu$mo $SO_4^{2-}$ accumulated in 30 min, using an initial mucosal $SO_4^{2-}$ concentration of 0.04 mM (1.0 mo per incubation).

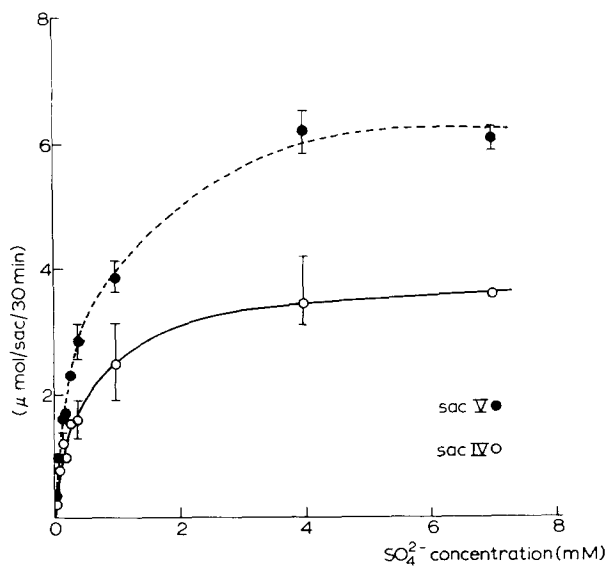


Fig. 2. $SO_4^{2-}$ transport by rat ileum. Dependence on $SO_4^{2-}$ concentration. Incubation as described under Methods. Each point is the mean of at least two determinations with range as shown. The range at lower concentration is normally too small to be visible. Initial $SO_4^{2-}$ concentration in the range 0.004–7.0 mM. Mucosal transfer is expressed as $\mu$mol $SO_4^{2-}$ accumulated in 30 min per sac. Mean sac length is 19.2$\pm$0.3 cm for sac V, 19.7$\pm$0.3 cm for sac IV. Mean sac weight is 1.37$\pm$0.04 g for sac V and 1.32$\pm$0.05 g for sac IV.

TABLE I

KINETIC CONSTANTS FOR SULPHATE TRANSPORT

Results calculated in three different ways and expressed ±S.E.

| Parameter | Units of measurement | | |
|---|---|---|---|
| | Sac | cm | g wet weight |
| Sac V | | | |
| $V$ (mmol/30 min) | 6.8 ±0.2 | 0.34±0.01 | 5.4 ±0.1 |
| $K_a$ (mM) | 0.60±0.05 | 0.61±0.08 | 0.69±0.06 |
| Sac IV | | | |
| $V$ (mmol/30 min) | 3.7 ±0.2 | 0.19±0.01 | 3.5 ±0.2 |
| $K_a$ (mM) | 0.44±0.08 | 0.46±0.07 | 0.75±0.10 |

substantially the same, and the fit of the data does not appear to be consistently better for any way of calculating. Results for sac IV expressed per g show a slight anomaly, and give somewhat higher values in most calculations. The length measurement in particular (the parameter with the apparently closest correspondence to the mucosal surface area) is, in practice, subject to variation in the degree of relaxation of the muscle layer [24]. When all animals are of very similar body weight, as here, there seems to be no disadvantage in the use of the simplest unit, the sac, and the remainder of the results are expressed in this way.

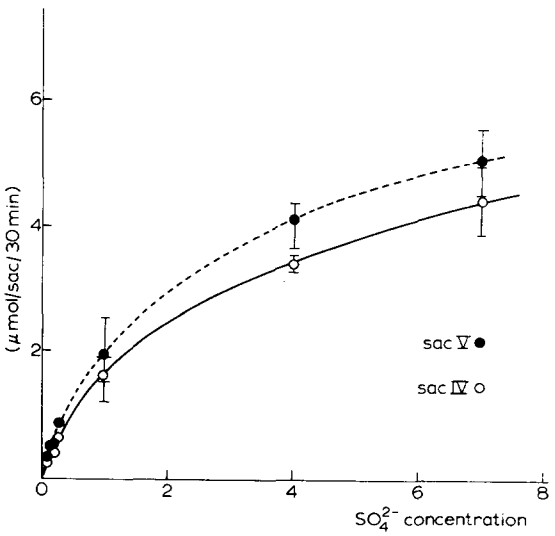


Fig. 3. $SO_4^{2-}$ transport by rat ileum in the presence of molybdate. Incubation as described under Methods. Each point is the mean of at least two determinations with range as shown. The range at lower concentrations is too small to be visible. Initial $SO_4^{2-}$ concentration in the range 0.1–7.0 mM. $MoO_4^{2-}$ concentration 1.0 mM throughout. Mucosal transfer is expressed as $\mu$mol $SO_4^{2-}$ accumulated in 30 min per sac. Mean sac length is 19.7±0.3 cm for sac V, 19.7±0.3 cm for sac IV. Mean sac weight is 1.37±0.06 g for sac V and 1.32±0.05 g for sac IV. These values agree well with those in the absence of $MoO_4^{2-}$ (legend to Fig. 2).

TABLE II

KINETIC CONSTANTS FOR MOLYBDATE INHIBITION

All data obtained using 1 mM sodium molybdate

| Parameter | Units of measurement | | |
|---|---|---|---|
| | Sac | cm | g wet weight |
| Sac V | | | |
| $V$ (mmol/30 min) | 7.2±0.7 | 0.34±0.02 | 5.2±0.4 |
| $K_a$ (mM) | 2.5±0.5 | 2.3 ±0.4 | 2.2±0.4 |
| $K_i$ (mM) | 0.31 | 0.36 | 0.46 |
| Sac IV | | | |
| $V$ (mmol/30 min) | 5.9±0.5 | 0.27±0.01 | 4.1±0.3 |
| $K_a$ (mM) | 2.6±0.5 | 2.1 ±0.3 | 1.8±0.3 |
| $K_i$ (mM) | 0.32 | 0.28 | 0.72 |

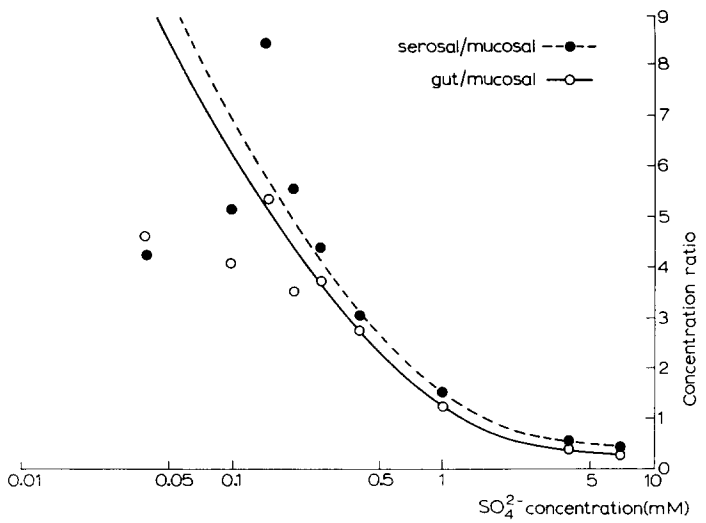


Fig. 4. Dependence on mucosal $SO_4^{2-}$ concentration of the degree of concentration of $SO_4^{2-}$ by rat lower ileum (sac V). Final serosal/mucosal and gut/mucosal concentration ratios developed are plotted against initial mucosal $SO_4^{2-}$ concentration. The values plotted are derived from the same experiments as those reported in Table I (for sac V). Final serosal, gut, and mucosal volumes were calculated from the weighings reported under Methods [22]. In calculating the gut concentration, the fluid content of the gut was assumed to be 80 % of the initial wet weight and this volume added to the gut fluid uptake. $SO_4^{2-}$ remaining in the mucosal fluid was determined directly by counting, but the final mucosal volume was obtained by difference. The logarithmic scale of the plot is purely for convenience and implies nothing about the relationship of the variables. The scatter of points at low concentrations is partly an effect of significant depletion of the mucosal fluid during incubation.

Fig. 2 shows the gut/mucosal and serosal/mucosal final concentration ratios as a function of initial mucosal concentration. The two ratios follow each other closely, suggesting that there is free diffusion between the gut wall and the serosal side. The high concentration ratios achieved after 30 min incubation show that there is no advantage in the long incubations of up to 3 h used by some authors [1, 3].

*Effect of $MoO_4^{2-}$ on $SO_4^{2-}$ transport*

A detailed study of the effect of $MoO_4^{2-}$ on the $SO_4^{2-}$ transport system was carried out by redetermining the kinetic parameters for $SO_4^{2-}$ transport in the presence of 1 mM $Na_2MoO_4$ (Fig. 3 and Table II). The fit to the Michaelis-Menten equation is again good, and $K_i$ for $MoO_4^{2-}$ in these conditions can be calculated from the data. Results are again expressed in three ways, and are not significantly different. There is no significant change in $V$ in the presence of $MoO_4^{2-}$, with particularly good agreement shown in the results for sac V, suggesting that the mode of inhibition is competitive [25]. The effect of $MoO_4^{2-}$ in vivo was examined in preliminary experiments with animals fed high levels of $Na_2MoO_4$ for periods of up to 6 weeks. Since the animals did not gain weight normally, individual control animals of closely similar weights were used. In every case $SO_4^{2-}$ transport was reduced in animals fed high $MoO_4^{2-}$ levels.

*Effect of other anions on $SO_4^{2-}$ transport*

Competitive inhibition of $SO_4^{2-}$ transport should not be confined to $MoO_4^{2-}$ but should be found for other oxyanions of same charge and stereochemistry and of similar size. The effects of $WO_4^{2-}$, $SeO_4^{2-}$ and $S_2O_3^{2-}$ have been examined at a single concentration, and all inhibit (Table III). Of other potential inhibitors, $CrO_4^{2-}$ is strongly oxidising, and $TeO_4^{2-}$ is not soluble under the conditions used. The effec of $SO_3^{2-}$ (Table III) is much smaller but still significant.

TABLE III

EFFECT OF OTHER ADDED ANIONS ON SULPHATE TRANSPORT

Sulphate concentration 0.27 mM; inhibitor anion concentration 1.0 mM (as sodium salt). Results of experiments are quoted as means ±S.E., with number of runs shown in parentheses.

| Added anion | Mucosal transfer (mmol/sac per 30 min) | |
|---|---|---|
| | Sac IV | Sac V |
| Control | 1.39±0.05 (8) | 2.15±0.15 (6) |
| $S_2O_3^{2-}$ | 0.33±0.02 (3) | 0.54±0.05 (3) |
| $SO_3^{2-}$ | 0.91±0.03 (3) | 1.19±0.14 (3) |
| $SeO_4^{2-}$ | 0.42±0.04 (3) | 0.80±0.04 (3) |
| $MoO_4^{2-}$ | 0.63±0.01 (2) | 0.81±0.03 (2) |
| $WO_4^{2-}$ | 0.55±0.10 (3) | 0.62±0.07 (3) |

*Specificity of inhibition*

The anions tested should be without effect on other transport systems. The specificity was checked by reference to the effect on L-valine transport, which is a well-characterised energy-dependent transport system [26]. Working at an L-valine concentration near to the $K_a$ value, no significant inhibition was found for any anion in sacs I, III or V. (Table IV). Inhibition is therefore probably not due to any interference with transport processes in general, but to specific competition for the binding site of $SO_4^{2-}$.

TABLE IV

EFFECT OF ANIONS ON L-VALINE TRANSPORT

These experiments were performed in normal $SO_4^{2-}$-containing Ringer solution, with inhibitor anion concentration, 1 mM; L-valine concentration, 3 mM. Results are expressed ±S.E. with the number of experiments in parentheses.

| Anion | Mucosal transfer (mmol/sac per 30 min) | | |
|---|---|---|---|
| | Sac I | Sac III | Sac V |
| Controls | 25.9±1.6 (4) | 38.6±1.4 (5) | 26.5±1.9 (5) |
| $MoO_4^{2-}$ | 28.4±1.6 (2) | 39.9±0.1 (2) | 24.3±0.3 (2) |
| $S_2O_3^{2-}$ | 24.9 | 34.5 | 27.8 |
| $WO_4^{2-}$ | 28.6 | 33.4 | 28.6 |
| $SeO_4^{2-}$ | | 31.2 | 25.6 |

*Fluid transport*

Fluid transport was measured in each experiment as an indication of the viability of the sacs. In experiments where $SO_4^{2-}$ at various concentrations was present in the medium without other added anions, mucosal fluid transport showed no dependence on $SO_4^{2-}$ concentration. Mean values for this parameter in this set of experiments are presented in Table V, with comparable published data [19], differing mainly in the use of a 1-h incubation time and a constant $SO_4^{2-}$ concentration. There is good agreement between the two sets of data. The presence of 1 mM $MoO_4^{2-}$, however, causes a small but significant decrease in fluid transport, again independent of $SO_4^{2-}$ concentration. There is no apparent relationship between the degree of inhibition of mucosal $SO_4^{2-}$ transport by $MoO_4^{2-}$ and the lowering of mucosal fluid transport. The effect of $MoO_4^{2-}$ on fluid transport is therefore a direct effect, and not a consequence of the reduction in $SO_4^{2-}$ transport. Fewer experiments were performed with other added anions. However, available data show no significant effect of $S_2O_3^{2-}$, $SeO_4^{2-}$, $WO_4^{2-}$ or $SO_3^{2-}$ on fluid transport at 1 mM.

TABLE V

FLUID TRANSPORT

Mean mucosal fluid transport was determined as described in ref. 19, although with a 30-min incubation time. The data have been calculated from experiments using a range of $SO_4^{2-}$ concentrations (0.004–7.0 mM) and are ±S.E. with the number of values in parentheses. Data from ref. 19 are quoted for comparison, but were only calculated by these authors in units of g initial wet weight of tissue.

| Conditions | Fluid transport ($g \cdot 30\ min^{-1}$) | | | |
|---|---|---|---|---|
| | Sac IV | | Sac V | |
| | Per sac | Per g | Per sac | Per g |
| Controls (no $MoO_4^{2-}$) | 1.37±0.06 (26) | 1.10±0.05 (26) | 1.05±0.03 (27) | 0.81±0.02 (27) |
| 1 mM $MoO_4^{2-}$ | 1.16±0.07 (14) | 0.91±0.05 (14) | 0.79±0.03 (14) | 0.59±0.03 (14) |
| Data from ref. 19 ($g \cdot h^{-1}$) | | 1.07±0.12 (10) | | 0.96±0.10 (10) |

## DISCUSSION

Wilson [6, 7], in early work with hamster intestine, reported that the chief site of $SO_4^{2-}$ uptake was in the first part of the intestine. All other reports, for rat [1, 2, 4, 8], mouse [5], guinea pig [4], hamster [1], rabbit [1, 3] and sheep (Cardin, C. J. and Mason, J., unpublished), as well as the present work, place the main site of $SO_4^{2-}$ absorption in the lower ileum. The results show that $SO_4^{2-}$ is concentrated by the intestine against a concentration gradient, in agreement with the results of Batt [5] for mouse ileum and those of Anast et al. [1] for rat, rabbit and hamster ileum. The serosal and tissue concentrations were generally similar at the end of incubation, suggesting that, as expected, the site of active concentration is the mucosal epithelium. Robinson [4] found some accumulation by rings of rat and guinea pig intestine, and we (Cardin, C. J. and Mason, J., unpublished) have evidence of accumulation by sheets of sheep ileum. $Cl^-$ is similarly accumulated by lower ileum in an energy-dependent process [27]. An energy requirement for $SO_4^{2-}$ transport had been previously demonstrated [1], and together with our kinetic results provide good evidence for the active transport of $SO_4^{2-}$. Our $K_a$ values for both sacs are close to 0.5 mM, an order of magnitude higher than the values reported for fungal systems [17].

$K_i$ for $MoO_4^{2-}$ inhibition is similar for both sacs IV and V, and is of the same order of magnitude as $K_a$ for $SO_4^{2-}$ in both sacs, i.e. about 0.5 mM. To establish the validity of the kinetic parameters, it is necessary to study $MoO_4^{2-}$ transport under the same conditions and the effect of $SO_4^{2-}$ on the system. These studies are in hand. An in vivo study of $MoO_4^{2-}$ uptake from rat intestine [28] showed that $MoO_4^{2-}$ was rapidly absorbed by the intestine, with duodenum > ileum > mid-section. We have shown that $MoO_4^{2-}$ and $SO_4^{2-}$ share a common transport system in the ileum, but one would expect the site of maximum absorption to be the same for each. Unpublished work from our laboratory suggests that, in vitro, maximum $MoO_4^{2-}$ uptake occurs in the ileum (Cardin, C. J. and Mason, J., unpublished; McKillen, M. and Fraser, K. I., personal communication).

Inhibition of $SO_4^{2-}$ transport by $S_2O_3^{2-}$, $SeO_4^{2-}$ and $WO_4^{2-}$ has been briefly reported in yeast [29], and a single energy-dependent permease was shown to transport $SO_4^{2-}$, $S_2O_3^{2-}$, $SeO_4^{2-}$ and $MoO_4^{2-}$ in species of penicillium and aspergillus [17]. In these studies the order of inhibition is the same as that found by us, viz. $S_2O_3^{2-} > SeO_4^{2-} \geqslant MoO_4^{2-} > WO_4^{2-}$, which corresponds to the order of decreasing anion size ($SO_4^{2-}$ is the smallest of all).

It has recently been shown that in rats fed a tungsten-supplemented diet, the levels of hepatic xanthine oxidase, sulphite oxidase and total molybdenum were reduced [30], implying the existence of a $WO_4^{2-}/MoO_4^{2-}$ competitive process. However, the competition is not only at intestinal absorption level, since intraperitoneal injections of tungsten also gave rise to reductions in these levels. A second noteworthy finding was that intestinal sulphite oxidase (a molybdoprotein) is located mainly in the lower ileum of the rat, equivalent to our sac V. The coincidence of maximum sulphite oxidase activity and maximum transport rate of the product of the oxidase reaction suggests that there might be a biochemical basis for the location of maximum $SO_4^{2-}$ transport in the lower ileum.

The work reported here supports the idea that the protective effect of $SO_4^{2-}$ against $MoO_4^{2-}$ toxicity in the rat is at least partly due to competition between the

two anions in the intestine for a common transport system, reducing $MoO_4^{2-}$ absorption. Some preliminary work (Cardin, C. J. and Mason, J., unpublished) with sheep ileum in vitro shows that $MoO_4^{2-}$ inhibits $SO_4^{2-}$ transport in this species too, though a common transport system has not yet been demonstrated. A second site where competition for a transport system may be important in both species is the renal tubule. Competition for reabsorption is indicated by studies in vivo [10].

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