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Sulphate transport in human placental brush-border membrane vesicles: competitive inhibition by selenate

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The effect of selenate on sulphate uptake by human placental brush-border membrane vesicles has been investigated. Selenate added to the incubation medium inhibits 1 mM sulphate uptake in a dose-dependent fashion with a K_i of approx. 2 5 mM. The inhibition by selenate is competitive, suggesting that selenate and sulphate share a common transporter (an anion exchange system) which may be of particular importance for the transport of such essential trace elements to the fetus.

Recent work has shown that sulphate transport in human placental brush-border membrane vesicles is mediated exclusively via anion exchange [1,2] Divalent anions such as chromate, molybdate, tungstate and thiosulphate have been found to be potent inhibitors of sulphate influx across this membrane when added to the incubation medium In contrast divalent anions such as phosphate, arsenate and tetraborate and monovalent anions including chloride, iodide and thiocyanate have been found to be weak inhibitors [2,3] One common feature of the potent inhibitory anions besides divalency is that they, like sulphate, possess a tetrahedral shape These anions may inhibit because they are competing for a common transport site with sulphate Bearing in mind that chromium and molybdenum are essential trace elements [4] these findings raise interesting questions regarding the nature of the transport of certain trace elements from the maternal to the fetal compartment We now report on experiments designed to investigate the effect of selenate on sulphate transport by human term placental brush-border membrane vesicles Selenate is a tetrahedral-shaped oxide of selenium which, like chromium and molybdenum, is an essential trace element [4]

Brush-border membrane vesicles were prepared from normal term human placentae by the calcium precipitation method of Boyd and Lund [5] Vesicles were suspended in a medium containing 1 mM K_2SO_4, 160 mM sucrose and 10 mM KOH-Hepes (pH 7 5) and stored at −70°C prior to use Sulphate uptake, using $^{35}SO_4^{2-}$ as a tracer, was assayed at 20°C using an ion-exchange column assay previously described by Shennan et al [6] Sulphate influx was determined after 2 min of incubation since this has been shown to be the approximate half-time of uptake [2]

Table I shows that 10 mM selenate or sulphate (added to the incubation medium as the sodium salt) inhibits the uptake of sulphate (1 mM) by approx 80% Also shown in Table I is the effect of 10 mM selenite this anion was found to be only a weak inhibitor This result is consistent with the earlier findings that only tetrahedral divalent anions are potent inhibitors of sulphate transport across this membrane The effect of varying extra-vesicular selenate over the concentration range 0 1 to 10 mM on 1 mM sulphate influx was tested Fig 1, a plot of sulphate uptake as a function of the logarithm of the selenate concentration, shows that selenate inhibits in a dose-dependent fashion with a K_i of approx 2 5 mM

We next tested to see if the inhibition by selenate of sulphate transport was competitive Sulphate uptake was measured from media containing 1, 2 5, 4 and 6 mM SO_4^{2-} in the presence and absence of 10 mM selenate We found that increasing the extravesicular SO_4^{2-} concentration reduces the proportion of sulphate influx which is inhibited by selenate A Lineweaver-Burk plot (Fig 2) shows that the K_t of SO_4^{2-} uptake is increased from 2 to approx 15 mM by the addition of 10 mM selenate but that V_{max} is unchanged A lower concentration of selenate (2 mM) added to the incubation medium similarly increases the K_t of sulphate uptake but does not alter V_{max} (results not shown) Thus it appears that selenate inhibits in a competitive manner

A comparison between these results and those of several studies on anion transport by rat ileum may be made Sulphate transfer across the everted sac has been shown [7] to be inhibited by the same anions that inhibit the membrane transport of sulphate into vesicles prepared from the maternal facing brush-border membrane of the human placenta Furthermore sulphate has also been found to inhibit the transport of selenate across rat ileum in vitro [8,9] One further similarity is that selenite is a much less effective inhibitor than selenate of sulphate transport in both tissues We have shown that the inhibition by selenate and the other potent anions (such as chromate, molybdate, tungstate, thiosulphate) of sulphate transport is on an anion exchange system whereas the studies on rat ileum do not give the locus of inhibition Anion exchange may thus be important for the intestinal absorption of selenium (in the form of selenate) and of other trace elements certainly

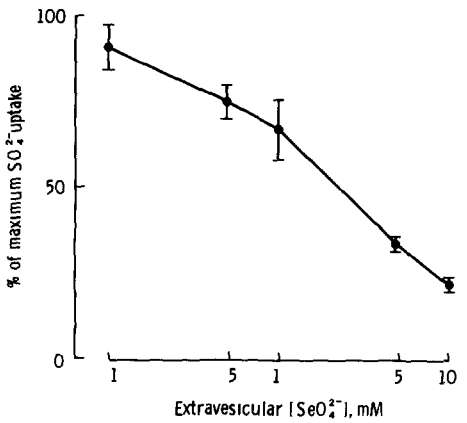

Fig 1 Effect of selenate concentration on sulphate uptake by human placental brush-border membrane vesicles Selenate was added to the incubation media as the Na salt over the range of 0 1–10 mM The incubation media also contained 1 mM K_2SO_4, sucrose (adjusted to maintain osmolarity) and 10 mM KOH-Hepes (pH 7 5) Note logarithmic scale of abscissa

TABLE I

THE EFFECT OF SELENATE, SULPHATE AND SELENITE ON SULPHATE UPTAKE BY HUMAN PLACENTAL BRUSH-BORDER MEMBRANE VESICLES

The control incubation medium contained 1 mM K_2SO_4, 190 mM sucrose and 10 mM KOH-Hepes (pH 7 5) When the inhibitors were required the medium containing 1 mM K_2SO_4, 160 mM sucrose, 10 mM Na_2X (where X = selenate, selenite or sulphate), and 10 mM Hepes-KOH (pH 7 5) Results are from three tissue preparations each studied in duplicate

Additional anion (10 mM)	SO^{2-} uptake (nmol/mg protein) (Mean ± S E)	% Inhibition
None (Control)	0 83 ± 0 06	–
Sulphate	0 16 ± 0 007	81
Selenate	0 14 ± 0 01	83
Selenite	0 70 ± 0 03	16

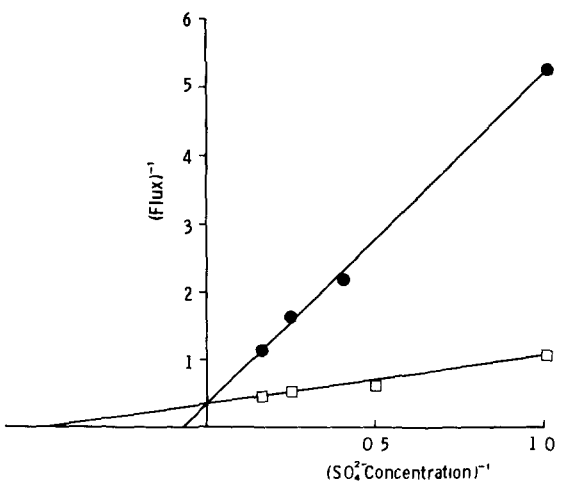

Fig 2 Double-reciprocal plot of sulphate uptake vs medium [SO_4^{2-}] in the presence (●) and absence (□) of 10 mM selenate Uptake was measured in media containing 1–6 mM SO_4^{2-} sucrose (concentration adjusted to maintain osmolarity), 10 mM KOH-Hepes (pH 7 5), with or without 10 mM sodium selenate

there is good evidence for anion exchange mechanisms in both the brush-border [10] and serosal-facing [11] membranes of this tissue However, with regard to intestinal absorption it is also possible that such tetrahedral divalent anions may be transported by the Na^+/SO_4^{2-} cotransporter which has been identified in the brush-border membrane of this tissue [12] but which is lacking in placenta [1,2]

In the placenta the finding that selenate competitively inhibits SO_4^{2-} transport into brush border membrane vesicles strongly suggests that selenate utilizes the anion exchange system and thus this mechanism may represent the pathway by which such trace elements gain access to the developing human fetus

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