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Sulfate Influx across the Rabbit Ileal Brush Border Membrane: Sodium and Proton Dependence, and Substrate Specificities

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Summary. In intact ileal mucosa, uptake of SO_4 across the brush border membrane requires the presence of Na and is saturable, with $K_{\frac{1}{2}} = 1.3$ mM at 140 mM Na (P.L. Smith, S.A. Orellana & M. Field, 1981. *J. Membrane Biol.* **63**:199–206). The present study examines the substrate specificities and transport stoichiometry of the Na-dependent SO_4 uptake process. The effects of variations in medium anion and cation composition on lumen-to-epithelium influx of SO_4 ($J_{me}^{SO_4}$) were determined under short-circuit conditions. $J_{me}^{SO_4}$ is inhibited by thiosulfate, but not by phosphate, methylsulfate, vanadate or taurocholate. Cl is weakly inhibitory. Uptake of SO_4 is poorly supported by Li, and is unaffected by K, indicating a specific dependence on Na. At low SO_4 concentration (0.22 mM), $J_{me}^{SO_4}$ is a hyperbolic function of medium Na concentration; the corresponding Hill plot is linear with a slope of 1.0, suggesting a transport stoichiometry of 1 Na:1 SO_4. At high SO_4 concentration (6.7 mM), the Na-dependent SO_4 velocity curve is sigmoidal and yields a Hill plot which is again linear but has a slope of 1.56, suggesting transport of more than 1 Na per SO_4. SO_4 uptake in presence of Na exhibits a dependence on medium pH. At 0.22 mM SO_4 and 140 mM Na, $J_{me}^{SO_4}$ was doubled by lowering pH from 7.4 to 6.8. However, at 6.7 mM SO_4 and 140 mM Na, changing pH had no effect on $J_{me}^{SO_4}$ over the range 6.8 to 8.5. The pH dependence of $J_{me}^{SO_4}$ at 6.7 mM SO_4 was restored when medium Na was lowered to 3 mM, suggesting that pH sensitivity is a function of the concentration of preformed $NaSO_4^-$ ion pair. The results suggest that SO_4 influx across the ileal brush border occurs by electroneutral $Na^+/NaSO_4^-$ or $Na^+/H^+/SO_4^{2-}$ cotransport, the former being favored by high concentrations of Na and SO_4.

Key words rabbit ileum · sulfate influx · sodium dependence · pH dependence · $NaSO_4^-$ ion pair · lithium

Introduction

Inorganic SO_4 is actively absorbed in rabbit distal ileum by an electroneutral process involving two separate transport steps: (i) Na-dependent influx across the brush border membrane [26], and (ii) efflux across the basolateral membrane by SO_4/Cl exchange [12]. Experiments with rat ileal brush border vesicles have shown that Na-dependent SO_4 influx is electrically neutral [16], suggesting a transport stoichiometry of 1 Na:1 X:1 SO_4, where X is Na^+ or another monovalent cation. The aim of the present study was to investigate in detail the SO_4 influx process; in particular, to define its anionic and cationic substrate specificities and to determine the transport stoichiometry of Na and SO_4. The results suggest a model for the brush border transport system in which electroneutral SO_4 influx occurs by either $Na^+/NaSO_4^-$ or $Na^+/H^+/SO_4^{2-}$ cotransport, depending on the relative concentrations of available substrate.

Materials and Methods

Materials

HEPES (N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid), HEPPS (N-2-hydroxypiperazine-N′-2-propane-sulfonic acid), PIPES (piperazine-NN′-bis-2-ethane-sulfonic acid), choline Cl and choline HCO_3 were obtained from Sigma (St. Louis, Mo.); (^{3}H)-PEG (mol wt 900), 4.5 mCi/mmol, and $H_2\,^{35}SO_4$, 40 mCi/ml (carrier-free) were obtained from New England Nuclear (Boston, Mass.).

Methods

Distal ileal mucosa was obtained from New Zealand white male rabbits which had been fed freely on a standard rabbit chow and water. The animals were killed by a blow to the neck. A section of terminal ileum was quickly excised, opened along its mesenteric border and rinsed clean of luminal contents with ice-cold standard Ringer's solution (*see below*). The tissue was stripped of serosa and muscularis by blunt dissection as previously described [7] and incubated in cold oxygenated Ringer's solution before mounting in chambers. Unless otherwise stated, the Ringer's solutions employed were modifications of one of the two following:

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(1) *Standard Ringer's* which consisted of (in mmol/liter): Na 142.6; K 5; Ca 1.25; Mg 1.32; Cl 123.7; HCO_3 25; PO_4 1.95; SO_4 0.22; and was bubbled with 95% O_2, 5% CO_2.

(2) *HCO_3-Free Ringer's* which consisted of (in mmol/liter): Na 139.6; K 5; Ca 1.25; Mg 1.32; Cl 145.7; PO_4 1.95; SO_4 0.22; HEPES (pK_a 7.55) 5; HEPPS (pK_a 8.0) 5; and PIPES (pK_a 6.8) 5; the solution was titrated to the desired pH with KOH and bubbled with 100% O_2.

In particular experiments, Na concentration was varied by substitution of choline Cl and choline HCO_3 for NaCl and $NaHCO_3$, and SO_4 concentrations were varied by isosmotic replacement of NaCl with Na_2SO_4 and mannitol, and of KCl with K_2SO_4 and mannitol. Details of any other solution alterations are given in the text and Figure legends.

Influx of SO_4 from the mucosal solution into the epithelium was determined under short-circuit conditions as previously described [26]. Briefly, tissues were mounted mucosa-side-up in multiport chambers and preincubated at 37° C in Ringer's solution of the desired composition for 30 to 40 min. The mucosal solution was then changed for a test solution containing 1 μCi $^{35}SO_4$ and 4 μCi (^{3}H)-PEG to which the tissues were exposed for 35 sec. The tissues were punched out, blotted, weighed and extracted overnight in 0.1 N HNO_3. SO_4 influx was calculated from the tracer content of the tissue extract after correcting for the extracellular contribution determined with (^{3}H)-PEG. Influxes are expressed in μmol/hr/g wet weight. (For purposes of comparison to earlier studies, the conversion factor for expression of fluxes in μmol/hr/cm^2 tissue surface area is on average 0.027.)

Results are generally expressed as means ±1 SE for n animals. For each animal a minimum of two ports was used for each control and experimental condition, and the individual flux rates for each port were averaged to give a mean value for that animal. Statistical comparisons, using Student's "t" test for paired variates, were made on the fluxes measured in individual ports so that the number of degrees of freedom is one less than the number of pairs of ports. The order of pairing was randomly assigned at the start of each experiment.

Results

Cation and Anion Specificities of SO_4 Influx

SO_4 influx across rabbit ileal brush border ($J_{me}^{SO_4}$) was previously shown to require the presence of Na in the luminal bathing medium: in Na-free choline Ringer's solution, no influx of SO_4 was detected [26]. We have investigated the cation specificity of the transport system by substituting Li and K for Na or choline (Table 1). Li (40 mM) slightly stimulated $J_{me}^{SO_4}$, but was less effective than 2 mM Na. $J_{me}^{SO_4}$ was unaffected by 30 mM K.

Table 2a shows the effects on $J_{me}^{SO_4}$ of a variety of mono-, di- and trivalent anions. (Medium SO_4 concentration = 0.22 mM.) Taurocholate (1 mM) had no effect on $J_{me}^{SO_4}$. Methylsulfate (1 mM) and vanadate (1 mM) were also without effect, as was divalent phosphate (2.1 mM). By contrast, thiosulfate (2.2 mM) inhibited SO_4 uptake by about 80%. The effects of the above anions were investigated using standard Cl, HCO_3-Ringer's solution as a control. As shown in Table 2b, Cl itself interacts with the SO_4 transport system to a modest degree. In Cl-free gluconate Ringer's, SO_4 influx was 65% higher than in Cl-Ringer's.

Table 1. Cation specificity of SO_4 influx[a]

Monovalent cations present (mM)				$J_{me}^{SO_4}$ (μmol/hr/g)
Na	Ch	K	Li	
a. Na vs. Choline:				
140	0	5	0	1.96±0.26 (19, 60)
0	140	5	0	0 (2, 7)[b]
b. Na vs. Li:				
40	100	5	0	1.55±0.11 (3, 6)
0	100	5	40	0.13±0.02 (6, 13)
2	138	5	0	0.17±0.03 (3, 6)
c. Na vs. K:				
10	135	0	0	0.28±0.04 (4, 11)
10	105	30	0	0.23±0.08 (4, 11)

[a] Values are means ±1 SE for (n, m) experiments where n = number of rabbits and m = number of ports. Tissues were bathed in Cl, HCO_3-Ringer's. SO_4 was present at 0.22 mM.
[b] Values taken from Smith et al. [26].

Table 2. Anion specificity of SO_4 influx

Test anion	$J_{me}^{SO_4}$, (μmol/hr/g)	
	Control	Experimental
a. Chloride-Ringer's:		
taurocholate, 1 mM (5, 16)	1.58±0.34	1.56±0.28
methylsulfate, 1 mM (3, 6)	1.55±0.56	1.45±0.18
phosphate, 2.7 mM (4, 9)	2.23±0.39	2.06±0.31
thiosulfate, 2.2 mM (3, 6)	2.23±0.39	0.48±0.07
vanadate, 1 mM (3, 6)	1.39±0.21	1.26±0.13
b. Gluconate-Ringer's:		
chloride, 120 mM (3, 12)	2.70±0.29	1.64±0.31

Values are means ±1 SE for paired experiments on tissues from (n, m) animals, where n = number of animals and m = number of ports.

a. Control fluxes were measured in standard Ringer's (SO_4 = 0.22 mM), except in PO_4 and S_2O_3 experiments, when control solutions contained no PO_4. All test anions were added to the mucosal-side solution only. With the exception of taurocholate, the test anions were present during both preincubation and flux periods. Taurocholate was added during the flux period only. (Note: At pH 7.4 and 2.7 mM PO_4: $(HPO_4^{2-}) = 2.1$ mM and $(H_2PO_4^-) = 0.6$ mM, correcting for ionic strength.)

b. Control fluxes were measured in standard Ringer's solution with 120 mM gluconate substituted for all Cl. In the experimental condition, gluconate was replaced by Cl in both bathing solutions and during both preincubation and flux periods.

Na-Dependence of SO_4 Influx

The nature of the Na-dependence of SO_4 uptake was examined by determining $J_{me}^{SO_4}$ at various con-

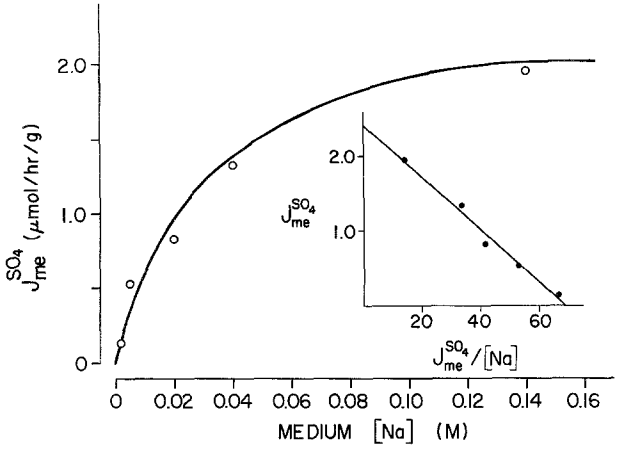

Fig. 1. SO_4 influx ($J_{me}^{SO_4}$) as a function of medium Na concentration at 0.22 mM medium SO_4. Tissues were bathed in standard Ringer's with the Na concentration varied by choline substitution. Each point represents the mean flux from 5 or more experiments (2–4 ports per experiment). For each experiment, solutions containing 140 mM Na were used as controls and the data have been normalized to the average control value of $J_{me}^{SO_4}$. Inset: Eadie-Hofstee plot of the data with the line drawn by linear regression analysis ($r=0.99$). $J_{max}^{SO_4}=2.435$ μmol/hr/g. $K_{\frac{1}{2}}=35$ mM

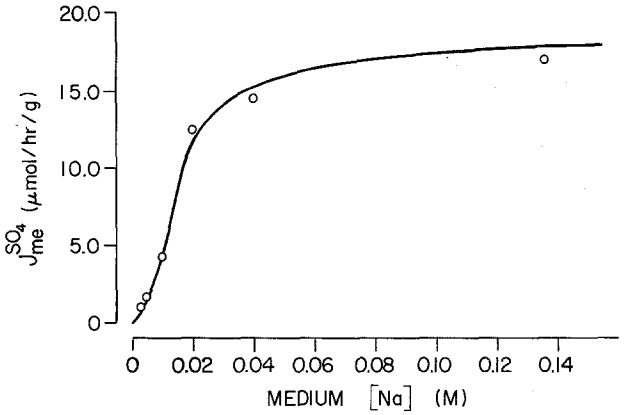

Fig. 2. SO_4 influx as a function of Na concentration at 6.7 mM medium SO_4. Each point represents the mean flux from 3 or more experiments (2–4 ports per experiment). *See* legend to Fig. 1 for further details

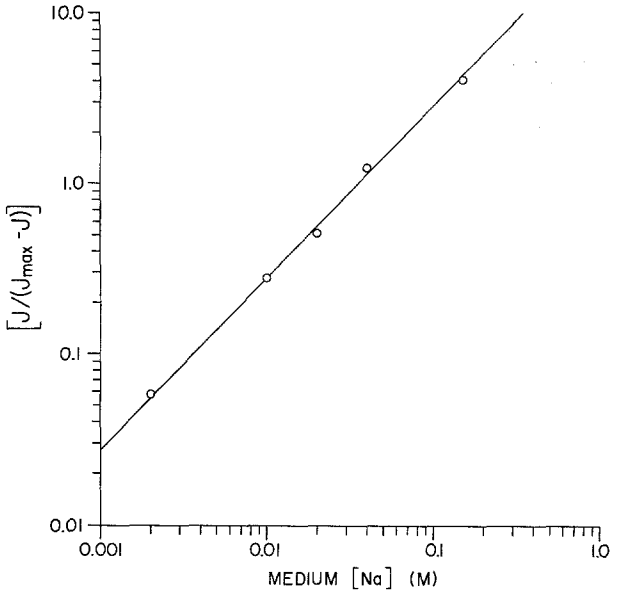

Fig. 3. Hill plot of SO_4 influx as a function of Na concentration at 0.22 mM medium SO_4. $J=J_{me}^{SO_4}$. $J_{max}=2.435$ μmol/hr/g, calculated from the Eadie-Hofstee plot of the data in Fig. 1. The line, drawn by linear regression analysis, has a slope (n value) of 1.008 ($r=0.999$)

centrations of medium Na, and at either low (0.22 mM) or high (6.7 mM) concentrations of SO_4. At 0.22 mM SO_4, $J_{me}^{SO_4}$ is a simple hyperbolic function of Na concentration, yielding a linear Eadie-Hofstee plot (Fig. 1). This linear transformation gives a $K_{\frac{1}{2}}$ value for Na of 35 mM and a maximum SO_4 influx rate $J_{max}^{SO_4}$ of 2.4 μmol/hr/g. At 6.7 mM SO_4, however, the relation between $J_{me}^{SO_4}$ and Na concentrations is sigmoidal and does not yield a linear Eadie-Hofstee plot (Fig. 2). Extrapolating directly from Fig. 2, $K_{\frac{1}{2}}$ and $J_{max}^{SO_4}$ for Na under these conditions are about 15 mM and 18 μmol/hr/g, respectively.

In order to estimate the stoichiometry of Na/SO_4 transport, the data from Fig. 1 and 2 were rearranged into the form of the Hill relationship (*see* Reference 24 for details):

$$\log \frac{J_{me}^{SO_4}}{J_{max}^{SO_4}-J_{me}^{SO_4}} = n \log Na - \log K_a.$$

The Hill plot at low SO_4 concentration (Fig. 3) is linear ($r=0.999$) with a slope (n) of 1.008, suggesting a transport stoichiometry of 1 Na:1 SO_4. At high SO_4 concentration (Fig. 4), the Hill plot again yields a straight line ($r=0.98$), but the slope in this case is 1.56, suggesting that more than one Na are transported with each SO_4.

pH-Dependence of SO_4 Influx

The influence of medium pH on SO_4 uptake was investigated by determining $J_{me}^{SO_4}$ in HCO_3-free Ringer's solutions bubbled with 100% O_2. pH was maintained at the desired level by means of a triple buffer system comprising HEPES (pK_a 7.55), HEPPS (pK_a 8.0) and PIPES (pK_a 6.8), each present at 5 mM. In a preliminary set of experiments with medium SO_4 concentration at 0.22 mM and Na at 40 mM, $J_{me}^{SO_4}$ showed a marked pH dependence (Table 3): lowering the pH of the bathing medium from 7.4 to 6.8 increased $J_{me}^{SO_4}$ by $\simeq 80\%$, while raising pH to 8.0 reduced $J_{me}^{SO_4}$ by $\simeq 40\%$. These results indicate that, in the presence of Na, protons stimulate the uptake of SO_4.

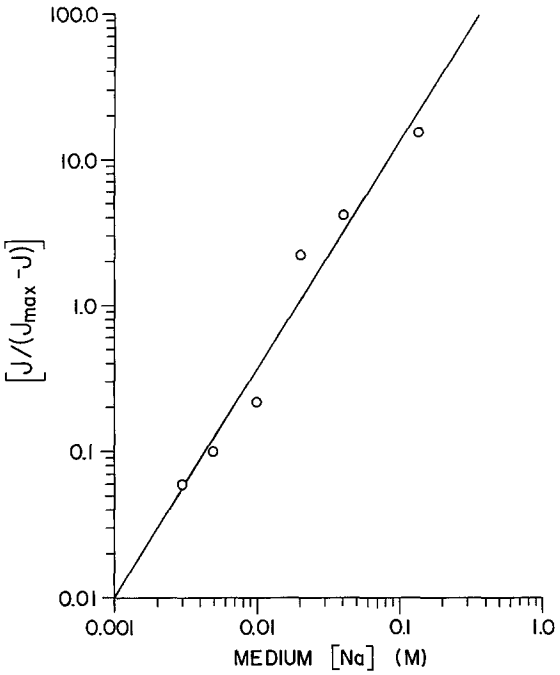

Fig. 4. Hill plot of SO_4 influx as a function of Na concentration at 6.7 mM medium SO_4. $J = J_{me}^{SO_4}$. J_{max} estimated as 18 μmol/hr/g from the data in Fig. 2. The line, drawn by linear regression analysis, has a slope (n value) of 1.56 ($r = 0.98$)

Table 3. Effect of medium pH on SO_4 influx

	$J_{me}^{SO_4}$ (μmol/hr/g)	
	Control (pH 7.4)	Experimental
pH 6.8 (3, 10)	0.71 ± 0.11	1.28 ± 0.11
pH 8.0 (4, 15)	0.56 ± 0.05	0.33 ± 0.04

Values are means ± 1 SE for paired experiments on (n, m) animals, where n = number of animals and m = number of ports. Tissues were bathed in HCO_3-free Ringer's solution containing 0.22 mM SO_4 and 40 mM Na. HEPES (pK_a 7.55); HEPPS (pK_a 8.0) and PIPES (pK_a 6.8) were added as buffers (concentration of each = 5 mM), and solutions were bubbled with 100% O_2. Experimental fluxes differ significantly from controls, $P < 0.01$. (Statistical comparison based on number of ports.)

Activation of SO_4 transport by protons does not by itself imply that H^+ and SO_4 are cotransported across the brush border membrane. For example, H^+ could act simply as a modifier of the transport system by increasing the affinity of the membrane transport site for SO_4. In order to establish cotransport, it would be necessary to demonstrate not only H^+-dependent SO_4 influx but also SO_4-dependent H^+ influx. If protons accompany SO_4 into the cell with a 1:1 stoichiometry, then at maximal SO_4 transport rates it should be possible to detect a pH change in the mucosal bathing medium. We thought it worthwhile to investigate this possibility despite the fact that the Hill coefficient for Na-dependence at high SO_4 concentration is greater than 1.0. The method at our avail is too insensitive to measure the proton flux which could be present at low SO_4 concentration. Accordingly, the following experiment was performed: using a HCO_3-free Ringer's solution and a slightly lowered pH (7.2) to limit ileal HCO_3 secretion [25], mucosal pH was monitored with a standard pH electrode before and after addition of SO_4 (to 6.7 mM)[1] to the mucosal bath. The serosal solution was buffered with 5 mM HEPES; the mucosal solution was buffered with only 0.1 mM HEPES so that at maximal $J_{me}^{SO_4}$, transport of 1 H^+ per 1 SO_4 should produce a detectable mucosal pH change of approximately 0.2 pH units over a period of 5 to 6 min. A representative experiment is shown in Fig. 5. In each of five experiments, no change in mucosal pH was seen following addition of SO_4.

Since the Na-dependent SO_4 velocity curves and corresponding Hill plots shown in Fig. 1–4 suggest that the Na:SO_4 transport stoichiometry may be different at high and low SO_4 concentrations, we undertook to investigate more systematically the pH-dependence of $J_{me}^{SO_4}$ (Table 4). With Na at the near-saturating concentration of 140 mM and SO_4 at 0.22 mM, lowering medium pH from 7.4 to 6.8 again almost doubled $J_{me}^{SO_4}$. However, at the same concentration of Na but at a high concentration of SO_4 (6.7 mM), changing pH over a wide range (6.8 to 8.5) had no effect on $J_{me}^{SO_4}$. A pH effect at high SO_4 concentration was seen, however, when Na concentration was lowered to 3 mM. Indeed, at this concentration of Na, the pH effect was most marked: lowering pH from 7.4 to 6.8 produced a four- to fivefold increase in $J_{me}^{SO_4}$. These results suggest that the influence of H^+ concentration is not dependent on the concentration of either SO_4 or Na alone, but rather is a function of the concentration of the ion pair, $NaSO_4^-$, as discussed below.

Discussion

The transport system for SO_4 uptake across the rabbit ileal brush border is saturable, requires the

[1] The SO_4 transport rate is maximal at 6.7 mM SO_4. To verify this, $J_{me}^{SO_4}$ was determined, at pH 7.4 and pH 6.8, in presence of 140 mM Na and either 6.7 mM or 12.7 mM SO_4. Increasing the SO_4 concentration to 12.7 mM did not further stimulate $J_{me}^{SO_4}$ (7 ports for each [SO_4] at each pH):

At pH 7.4: $J_{me}^{SO_4} = 13.92$ (6.7 mM SO_4) and 13.91 (12.7 mM SO_4) μmol/hr/g.

At pH 6.8: $J_{me}^{SO_4} = 16.26$ (6.7 mM SO_4) and 15.42 (12.7 mM SO_4) μmol/hr/g.

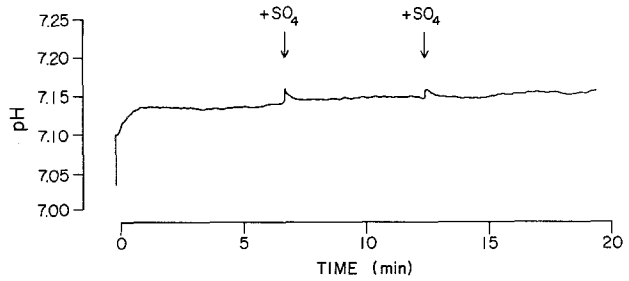

Fig. 5. Lack of effect of mucosal SO_4 on proton transport. Tissues were bathed in SO_4-free, HCO_3-free Ringer's solution and bubbled with 100% O_2. The mucosal solution was buffered with 0.1 mM HEPES and the serosal solution with 5 mM HEPES; both solutions were maintained at approximately pH 7.2. At this pH there is no spontaneous HCO_3 secretion [25]. Mucosal pH was monitored using a standard glass pH electrode (Corning Semimicro Combination electrode #476050). After a period of stabilization, a bolus of SO_4 (to yield 6.7 mM) dissolved in the mucosal Ringer's solution (pH 7.2) was added to the mucosal side. If H^+ is cotransported with SO_4 with a 1:1 stoichiometry, addition of SO_4 should have produced a detectable pH change in the mucosal solution of 0.2 pH units over 5 to 6 min, calculated from the measured $J_{me}^{SO_4}$ at 6.7 mM SO_4 and 140 mM Na, and the buffering capacity of 0.1 mM HEPES at pH 7.2. Shown above is one representative tracing: no mucosal pH change was detected in five such experiments

Table 4. pH dependence of SO_4 influx at different Na and SO_4 concentrations

Conditions			$J_{me}^{SO}4$ (μmol/hr/g)	
[SO_4]	[Na]	pH	Control (pH 7.4)	Experimental
0.22	140	6.8	1.24±0.38	2.14±0.62
6.7	140	6.8	16.45±2.18	16.08±2.72
6.7	140	8.5	15.85±2.82	15.93±2.31
6.7	003	6.8	1.08±0.62	5.28±0.45

Values are means ±1 SE for paired experiments on tissues from 3 rabbits and 10 or 11 ports. Tissues were bathed in HCO_3^--free Ringer's bubbled with 100% O_2. HEPES, HEPPS and PIPES (5 mM each) were present as buffers. In the 3rd row (pH 8.5), only the mucosal pH was altered, the serosal pH being kept at 7.4. In the other rows, both mucosal and serosal pHs were altered.

presence of luminal Na, and leads to intracellular accumulation of SO_4 above electrochemical equilibrium [26]. These features are most readily explained in terms of a carrier-mediated system which effects cotransport of both Na and SO_4 and is energetically coupled to the Na gradient. This model of secondary active transport has been widely accepted as the basis for Na-dependent intestinal transport of substrates other than SO_4; for example, sugars [4], amino-acids [5], bile salts [15] and Cl [19]. In the present study, SO_4 influx is shown to satisfy two of the principal criteria for such carrier-mediated cotransport processes. Firstly, the Na-dependence of SO_4 uptake exhibits saturation kinetics, $J_{me}^{SO_4}$ being a saturable function of medium Na concentration at both high and low concentrations of SO_4. Secondly, the SO_4 transport system shows a marked degree of both cationic and anionic substrate specificity.

SO_4 influx, which is undetectable in Na-free choline Ringer's [26], is only weakly supported by Li and is unaffected by K. A partial substitution of Na by Li has been observed in a variety of intestinal Na-coupled transport processes [23]. In ileal brush border vesicles from the rat, Lücke and associates [16] observed stimulation of SO_4 uptake, and the typical gradient "overshoot" phenomenon, only in presence of Na; K, Rb, Cs and also Li were without effect. Our observed stimulation of SO_4 influx by Li was so slight that it could easily have been missed in the vesicle studies.

Appropriate charge and stereospecificity appear to be important requirements for the anionic substrate of the cotransport system. $J_{me}^{SO_4}$ was unaffected by divalent phosphate, methylsulfate and vanadate, and also by the bile salt taurocholate, which, like SO_4, is absorbed in the terminal ileum by a Na-dependent process [14].[2] By contrast, the presence of thiosulfate ($S_2O_3^{2-}$) markedly reduced $J_{me}^{SO_4}$, suggesting that this anion may be transported via the same cotransport system. Cardin and Mason [3], using everted sacs from rat ileum, found that SO_4 transport was inhibited by a series of oxyanions of the same charge and stereospecificity, in the order $S_2O_3^{2-} > MoO_4^{2-} > WO_4^{2-}$. Similarly, in rat ileal brush border vesicles, trans-stimulation of Na-dependent SO_4 transport occurs with MoO_4^{2-} but not with PO_4^{2-} [16].

Na:SO_4 Stoichiometry

Experiments with rat ileal brush border vesicles have shown that Na-dependent SO_4 uptake is electrically neutral [16]. Measurements of potential difference across intact ileal mucosa confirm the electroneutrality of SO_4 transport, at least a high SO_4 concentrations [26]. These findings suggest that SO_4 influx has a stoichiometry of either 2 Na: 1 SO_4, or 1 Na:1X:1 SO_4, where X is another monovalent cation. In the present study, the Na-dependence of SO_4 uptake was investigated at both low (0.22 mM) and high (6.7 mM) concentrations

[2] Taurocholate uptake in guinea pig ileal brush border vesicles is unaffected by the presence of SO_4[20].

of medium SO_4, and the Na/SO_4 stoichiometry was calculated by generating Hill plots of the data. At 0.22 mM SO_4, the simple hyperbolic relationship between $J_{me}^{SO_4}$ and Na concentration and the Hill coefficient of 1.0 suggest that 1 Na is transported per SO_4 and, therefore, in view of the electroneutrality, that another cation is also transported. At 6.7 mM SO_4, however, the relationship between $J_{me}^{SO_4}$ and Na concentration is sigmoidal which implies transport of more than one Na with each SO_4. The corresponding Hill plot has a slope of 1.56, confirming the sigmoidicity of the velocity curve and suggesting a transport stoichiometry of 2 Na per SO_4, with cooperativity between the two Na binding sites. Consistent with such cooperativity is the higher affinity for Na observed at 6.7 mM SO_4 ($K_{\frac{1}{2}}=15$ mM) compared with that noted at 0.22 mM SO_4 ($K_{\frac{1}{2}}=35$ mM).

pH Dependence

The dependence of SO_4 influx observed in the present study indicates conditions under which protons as well as Na activate SO_4 uptake, and therefore raises the possibility that a proton might accompany 1 Na and 1 SO_4 across the brush border membrane. Measurement of a SO_4-dependent H^+ flux could only be attempted, unfortunately, at maximal rates of SO_4 transport. At appreciably lower rates of transport, the estimated H^+ flux would be too small to detect by a pH change in the bathing solution. At a maximal SO_4 transport rate, no evidence was found for H^+ movement into the cell. While this argues against $Na/H/SO_4$ cotransport at high SO_4 and Na concentrations, it does not exclude the possibilities that a) rapid rectification of H^+ concentration via other pathways (e.g., Na^+/H^+ exchange) prevents detection of a mucosal pH change, or b) a pH effect is not seen because the proton concentration is not rate limiting under these substrate conditions. For example, H^+ binding to the carrier could increase the affinity for SO_4 binding with the result that there is no pH effect with SO_4 at saturating concentration; similarly, SO_4 binding could cause a shift in the pK for proton binding such that at high SO_4 concentration the carrier is already H^+-saturated at pH 7.4. However, additional evidence against $Na/H/SO_4$ cotransport at high SO_4 and Na concentrations is provided by the Na-dependence data which suggest 2 Na per SO_4 cotransport under these conditions.

The possibility remains of course that a proton might be cotransported when the relative concentrations of the three substrates are different. In examining this possibility, we found that when both SO_4 and Na concentrations are high, $J_{me}^{SO_4}$ is independent of pH over a wide range (6.8 to 8.5). When either SO_4 concentration or Na concentration is low, however, the pH effect is restored. Thus, the effect of pH clearly does not depend on the concentration of SO_4 or of Na alone, since it is present at both high and low concentrations of both species. Changing the concentration of Na and SO_4 alters not only the concentration of the free ions but also of the ion pair complex, $NaSO_4^-$. The distribution of free and complexed species in electrolyte solutions is an important determinant of the thermodynamic properties of such solutions. Calculation of the species distribution in a mixed electrolyte solution requires the use of complex computerized chemical models to overcome the problem of solving simultaneous chemical equilibria. However, an estimate of the distribution of free and complexed Na and SO_4 can be formulated from the expressions for mass balance conditions and the stability constant equation, $K_{eq}=[NaSO_4^-]/[Na][SO_4]$. The stability constant ($\log K_{eq}$) for $NaSO_4^-$ at 37 °C is 0.75 (*see* Serial Publication No. 17 of the Chemical Society, London, 1964). This simple approach ignores the competition for SO_4 ion pair formation from other cations in the solution; however, it is clear that such competition will be most pronounced at low Na concentration, so that the estimated $NaSO_4^-$ concentration for these conditions will be considerably higher than the true concentration. Table 5 shows the pH dependence of $J_{me}^{SO_4}$ at different concentrations of $NaSO_4^-$. There is a rough correlation between the magnitude of the pH effect and the concentration of the ion pair.

Table 5. Correlation between $NaSO_4^-$ concentration and the pH effect on SO_4 influx[a]

SO_4 and Na concentration (mM)	$NaSO_4^-$ concentration (mM)	$J_{me}^{SO_4}$ (pH 6.8)/$J_{me}^{SO_4}$ (pH 7.4)
$SO_4=6.7$, $Na=140$	2.9	1.0
$SO_4=0.22$, $Na=140$	0.1	1.7
$SO_4=6.7$, $Na=3.0$	<0.1[b]	4.9

[a] Effect of pH on $J_{me}^{SO_4}$ at different concentrations of $NaSO_4^-$ ion pair. *See* text for details.

[b] At 3 mM Na, competition for $NaSO_4^-$ ion pair formation from other cations in the solution would be much greater than at 140 mM Na, so that 0.1 mM $NaSO_4^-$ concentration represents a considerable overestimate in this case.

Interactions Between Na, SO_4 and H^+ and the Membrane Transporter

By considering the effects of alterations in Na, SO_4 and H^+ concentrations on the rate of SO_4 influx, it should be possible to determine whether the three substrates interact with the membrane transporter separately or together, as preformed ion complexes.

In any carrier-mediated transport system there must be both a finite carrier population density and a finite rate of carrier turnover, so that at any given time a finite number of membrane transport sites are available to the substrate(s). This phenomenon underlies the saturability characteristic of carrier-mediated transport processes, and has the consequence that if the carrier is saturated with one of a pair of substrates, increasing the concentration of the other will stimulate the flux only if the two substrates bind separately to the transporter. If, on the other hand, the two substrates bind to the carrier as an ion pair (preformed in aqueous solution) and the carrier is saturated with one of the paired substrates, then increasing the concentration of the other will not alter the flux rate. Although the Na/SO_4 cotransport system is SO_4-saturated at 6.7 mM (*see* footnote 1), increasing Na concentration at this SO_4 concentration stimulated $J_{me}^{SO_4}$. Similarly, with Na at a near-saturating concentration of 140 mM, increasing SO_4 concentration greatly enhanced $J_{me}^{SO_4}$. These observations suggest that there is at least one site on the membrane transporter at which Na binds separately from SO_4, and not as the ion pair $NaSO_4^-$.

The same argument can be applied to the effect of changes in medium pH. The marked stimulation of $J_{me}^{SO_4}$ observed with increasing H^+ concentration when SO_4 is present at a saturating concentration of 6.7 mM (Na = 3 mM) suggests that H^+ and SO_4 do not bind to the membrane transporter as the performed ion pair HSO_4^-. Indeed, for a transport model in which H^+ and SO_4 bind as HSO_4^- ion pair, one would have to accept an exceedingly high affinity of the transporter for HSO_4^-: since medium SO_4 concentration is proportional to medium HSO_4^- concentration at constant pH, the $K_{\frac{1}{2}}$ for HSO_4^- would be about 6 nM (assuming a $K_{\frac{1}{2}}$ for SO_4 of 1.3 mM from the data of Smith et al. [26], and a pK for HSO_4^- of 2.0).

Model for SO_4 Transport across the Ileal Brush Border

The principal findings of the present study may be summarized as follows: (i) SO_4 uptake has a specific requirement for Na (Table 1); (ii) the Na:SO_4 transport stoichiometry is different at high and low concentrations of SO_4 (Fig. 1–4); (iii) SO_4 uptake is stimulated by protons in the presence of Na (Table 3), and (iv) changing the concentrations of Na and SO_4 alters the magnitude of the pH effect; the latter, however, is not simply a function of either SO_4 or Na concentration alone (Table 4). On the basis of these results, we propose the model for trans-brush border uptake of SO_4 depicted in Fig. 6. The model indicates two separate binding sites on the membrane carrier:

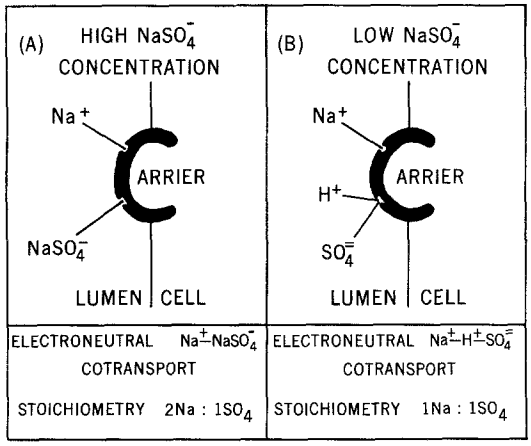

Fig. 6. Model for the trans-brush border uptake of SO_4. *See* text for details

1) *Site 1* – binds Na^+;

2) *Site 2* – binds either $NaSO_4^-$ ion pair, or H^+ and SO_4^{2-} (these ions binding separately and not as the ion pair HSO_4^-): the relative concentrations of $NaSO_4^-$ and of H^+ and SO_4^{2-} will determine which of the substrates bind, as well as the relative affinities of *Site 2* for the ion pair and for H^+ and SO_4^{2-}. When the availability of $NaSO_4^-$ is high (Fig. 6*A*), *Site 2* will be occupied by the ion pair, and changing H^+ concentration over a wide range will not affect $J_{me}^{SO_4}$; the net result is electroneutral $Na^+/NaSO_4^-$ cotransport (i.e., 2 Na:1 SO_4). When the availability of $NaSO_4^-$ is low (Fig. 6*B*), *Site 2* can bind H^+ and SO_4^{2-}, thus greatly expanding the capacity of the transport system for SO_4 uptake; the net result is electroneutral $Na^+/H^+/SO_4^{2-}$ cotransport (i.e., 1 Na:1 H^+: 1 SO_4).

Significance and Relations to Studies in Other Systems

An association between proton and SO_4 transport would not be unique to the ileum. In mitochondria, SO_4 uptake appears to occur by H^+ symport [21]. In the red blood cell anion exchange system, protons and SO_4 can be cotransported across the

cell membrane in exchange for Cl [10]. Milanick and Gunn [17] have recently shown that H^+ and SO_4^{2-} bind separately to the erythrocyte membrane transporter and not as the preformed ion pair HSO_4^-; their data are consistent with a random order of binding, with the binding of the first substrate increasing the affinity of the transporter for the second substrate.

When $NaSO_4^-$ availability is high, this ion pair appears to be transported along with a Na^+ ion into the ileal epithelial cell. Again a precedent for the transport of combinations of monovalent cations and divalent anions exists in the red cell, where the anion exchange system can carry $NaCO_3^-$ and $LiCO_3^-$ and probably other ion combinations [6, 8, 29].

In marked contrast to the erythrocyte anion exchange system, which can transport a wide range of substrates [11], the ileal brush border transport for SO_4 is a highly specific system, not shared by other anionic species. This is attested to by the lack of any inhibitory effect on SO_4 uptake by a variety of anions, including PO_4^{2-} and the bile salt taurocholate, and by the very modest inhibition by high concentrations of Cl. In this context, it is interesting to note that the transport systems for SO_4 in the ileum and in the renal tubule show striking similarities. The kidney has a specific mechanism for handling inorganic SO_4 by means of which plasma SO_4 concentration is maintained within fairly narrow limits (e.g., in dog −1.2 to 1.8 mmol/liter). This mechanism displays the following features: electroneutral Na-dependent influx across the brush border membrane [5, 22, 27]; lack of effect of PO_4^{2-} and Cl on brush border uptake [1, 13, 15]; SITS-inhibitable anion exchange at the basolateral membrane [1, 9]; and competition for transport by $S_2O_3^{2-}$ and MoO_4^{2-}, two anions of the same charge and stereospecificity as SO_4[1, 2, 15, 28]. The influence of protons on luminal SO_4 uptake in the renal tubule has not been specifically examined, although Lücke and colleagues [15] mention, without showing data, that the SO_4 transport system in the luminal membrane does not show a high pH sensitivity.

A major use for inorganic SO_4 is in enzyme-catalyzed sulfation or sulfoconjugation reactions which take place in a variety of tissues. Studies of sulfate conjugation of drugs in the rat liver suggest that the cellular pool of SO_4 is very small and that SO_4 is taken up from the plasma when required for sulfoconjugation [18]. Fine control of the circulating plasma concentration of SO_4 may therefore be important to biological functions involving this inorganic anion. The high degree of specificity of the SO_4 transport system in the ileum and the marked similarity of its properties to those of renal SO_4 transport, suggest that the ileum, as well as the kidney, may play a significant role in the maintenance of a steady plasma SO_4 concentration.

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