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Sulfate Transport in Rabbit Ileum: Characterization of the Serosal Border Anion Exchange Process

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Summary. The preceding paper [30] shows that transepithelial ileal SO_4 transport involves Na-dependent uptake across the ileal brush border, and Cl-dependent efflux across the serosal border. The present study examines more closely the serosal efflux process. Transepithelial mucosa (*m*)-to-serosa (*s*) and *s*-to-*m* fluxes (J_{ms}, J_{sm}) across rabbit ileal mucosa were determined under short-circuit conditions. SO_4 was present at 0.22 mM. In standard Cl, HCO_3 Ringer's, $J_{ms}^{SO_4}$ was 81.3 ± 5.3 (1 SE) and $J_{sm}^{SO_4}$ was 2.5 ± 0.2 nmol $cm^{-2} hr^{-1}$ ($n = 20$). Serosal addition of 4-acetamido-4′-isothiocyanostilbene-22′-disulfonate (SITS), 44′-diisothiocyanostilbene-22′-disulfonate (DIDS) or 1-anilino-8-naphthalene-sulfonate (ANS) inhibited SO_4 transport, SITS being the most potent. Several other inhibitors of anion exchange in erythrocytes and other cells had no effect on ileal SO_4 fluxes. In contrast to its effect on SO_4 transport, SITS (500 μM) did not detectably alter Cl transport.

Replacement of all Cl, HCO_3 and PO_4 with gluconate reduced $J_{ms}^{SO_4}$ by 70% and increased $J_{sm}^{SO_4}$ by 400%. A small but significant $J_{net}^{SO_4}$ remained. $J_{ms}^{SO_4}$ could be increased by addition to the serosal side of Cl, Br, I, NO_3 or SO_4. The stimulatory effect of all these anions was saturable and SITS-inhibitable. The maximal $J_{ms}^{SO_4}$ in the presence of Cl was considerably higher than in the presence of SO_4 (73.1 and 42.2 nmol cm^{-2} hr^{-1}, respectively; $p < 0.001$). The $K_{\frac{1}{2}}$ value for Cl was 7.4 mM, 10-fold higher than that for SO_4 (0.7 mM). Omitting HCO_3 and PO_4 had no measurable effects on SO_4 fluxes.

This study shows that (*i*) SO_4 crosses the serosal border of rabbit ileal mucosa by anion exchange; (*ii*) the exchange process is inhibited by SITS, DIDS and ANS, but not by several other inhibitors of anion exchange in other systems; (*iii*) SO_4 may exchange for Cl, Br, I, NO_3 and SO_4 itself, but probably not for HCO_3 or PO_4; (*iv*) kinetics of the exchange system suggest there is a greater affinity for SO_4 than for Cl, although the maximal rate of exchange is higher in the presence of Cl; and, finally (*v*) SITS has little or no effect on net Cl transport.

Key words: rabbit ileum, sulfate transport, anion exchange

The preceding paper [30] shows that active SO_4 absorption in rabbit ileum involves two separate steps: (*i*) a Na-dependent uptake at the brush border, and (*ii*) a Cl-dependent efflux at the serosal border. The latter can be blocked by the stilbene derivatives 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS)[1] and 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS), which are potent inhibitors of anion exchange in both erythrocytes [5] and Ehrlich ascites tumor cells [22, 23]. In the present study, we further investigate the serosal border component of SO_4 transport in rabbit ileum. Transepithelial mucosa (*m*)-to-serosa (*s*) and *s*-to-*m* fluxes of SO_4 were determined under short-circuit conditions in Ringer's solutions of different anionic compositions. The effects of a number of anion transport inhibitors were also tested. For most flux measurements, the SO_4 concentration was set at 0.22 mM, or about 10-fold lower than in the preceding study [30], so that SO_4 would be present mainly as a tracer and would not appreciably alter intracellular concentrations of other ions. The results provide additional evidence that anion exchange is the means by which SO_4 crosses the basolateral membrane of the ileal epithelial cell and define the anionic specificities of this exchange. While qualitative similarities between this anion exchange system and that

[1] The following abbreviations are employed: SITS: 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate; DIDS: 4,4′-diisothiocyanostilbene-2,2′-disulfonate; DNDS: 4,4′-dinitrostilbene-2,2′-disulfonate; DADS: 4,4′-diaminostilbene-2,2′-disulfonate; ANS: 1-anilino-8-naphthalene-sulfonate; NAP-taurine: N-(4-azido-2-nitrophenyl)-2-aminoethylsulfonate; HEPES: N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid.

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of red blood cells were found, there were also marked differences in substrate and inhibitor specificities.

Materials and Methods

Materials

Na, Ca, K and Mg salts of gluconic acid, and HEPES: Sigma Chemical Company (St. Louis, Mo.), SITS and DIDS: Pierce Chemical Company (Rockford, Ill.); DNDS, DADS and phloretin: K & K Laboratories (Cleveland, Ohio); Furosemide: Hoechst Pharmaceuticals, Inc. (Cincinnati, Ohio); Ethacrynic acid: Merck & Co., Inc. (Rahway, N.J.); Dipyridamole: Boehringer Ingelheim Ltd. (Elmsford, N.Y.); $Na_2{}^{35}SO_4$ (691.9 mCi/mmol) and $H^{36}Cl$ (5.9 mCi/g): New England Nuclear (Boston, Mass.).

Methods

Procedures for obtaining and mounting rabbit ileal mucosa in Ussing chambers and for measuring short-circuit current (I_{sc}), tissue conductance (G_t), and unidirectional mucosa (*m*)-to-serosa (*s*) and *s*-to-*m* fluxes of SO_4 and Cl have been described previously [10]. The Ringer's solutions employed were based on two stock solutions:

(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) *Gluconate Ringer's* which consisted of (in mmol/liter) HEPES, 3; Na, 140; K, 5; Ca, 1.25; Mg, 0.22; gluconate, 147.5; SO_4, 0.22, and was gassed with 100% O_2.

Unless otherwise stated, the pH of both solutions was 7.4. The anionic composition of the gluconate Ringer's was varied in particular experiments by substituting Cl, Br, I, NO_3, PO_4, HCO_3 or SO_4 for gluconate (in the case of SO_4, mannitol was used to balance osmolarity). Glucose (10 mmol/liter) was routinely added to the serosal medium and an equimolar amount of mannitol was added to the mucosal medium.

Results

Anionic Specificities

Unidirectional and net fluxes of SO_4 measured at a medium concentration of 0.22 mM are shown in Table 1. In standard Ringer's, *m*-to-*s* and *s*-to-*m* SO_4 fluxes were 81 and 2.5 nmol cm^{-2} hr^{-1}, respectively, yielding a net flux of 79; replacing inorganic anions (Cl, HCO_3 and PO_4) with gluconate reduced the *m*-to-*s* flux to 25 and increased the *s*-to-*m* flux to 10, yielding a net flux of 15. I_{sc} was the same in both Ringer's solutions but G_t was about 25% lower in gluconate Ringer's. In both standard and gluconate Ringer's, therefore, fluxes measured at 0.22 mM SO_4 are qualitatively similar to those observed in the preceding paper at a 10-fold higher SO_4 concentration.

Although replacement of Cl with gluconate reduces the transepithelial *m*-to-*s* and net SO_4 fluxes, it does not diminish the brush border influx of SO_4 [30]. It appears, therefore, that SO_4 crosses the basolateral border of the ileal epithelial cell by anion exchange, and that it is this exchange process which is interfered with when inorganic anions in the medium are replaced by gluconate. In order to investigate the anionic specificities of this exchange mechanism, *m*-to-*s* SO_4 fluxes were determined at various concentrations of each of several anions. The test anions were substituted for gluconate in gluconate Ringer's. In the case of SO_4 substitutions, only serosal SO_4 concentration was varied, the mucosal concentration being kept at 0.22 mM. With all other ionic substitutions, concentrations of the substituted anions were equal in both mucosal and serosal media. As shown in Fig. 1, the *m*-to-*s* SO_4 flux is stimulated by serosal SO_4, the effect being saturable and consistent with simple Michaelis-Menten kinetics (J_{max} = 42.2 nmol cm^{-2} hr^{-1} and $K_{\frac{1}{2}}$ = 0.7 mM). Figure 2 shows the stimulatory effect of Cl on *m*-to-*s* SO_4 flux (J_{max} = 73.1 and $K_{\frac{1}{2}}$ = 7.4); the $K_{\frac{1}{2}}$ and J_{max} of SO_4/Cl

Table 1. SO_4 fluxes, short-circuit current and conductance in standard and gluconate Ringer's solutions: Effect of SITS in gluconate Ringer's

Ringer's solution	$J_{ms}^{SO_4}$	$J_{sm}^{SO_4}$	$J_{net}^{SO_4}$	I_{sc}	G_t
Standard (20)	81.3 ± 5.3	2.5 ± 0.2	78.7 ± 5.4	1.5 ± 0.2	30.4 ± 1.0
Gluconate (12)	24.6 ± 1.0	10.2 ± 0.6	14.5 ± 1.0	1.3 ± 0.1	22.6 ± 0.5
Gluconate + 500 μM SITS (3)	4.5 ± 0.8	2.2 ± 0.4	2.4 ± 0.8	1.3 ± 0.1	22.2 ± 1.1

SO_4 fluxes are in nmol cm^{-2} hr^{-1}, I_{sc} in μEq cm^{-2} hr^{-1} and G_t in mmho cm^{-2}. Values are means ± 1 SE for paired experiments on tissues from (*n*) animals. SO_4 concentration in both Ringer's solutions was 0.22 mM; the gluconate Ringer's was free of Cl, HCO_3 and PO_4 (*see* Methods for exact composition). SITS was added to the serosal medium (mean control values for paired SITS experiments were no different from those shown for the larger gluconate Ringer's group).

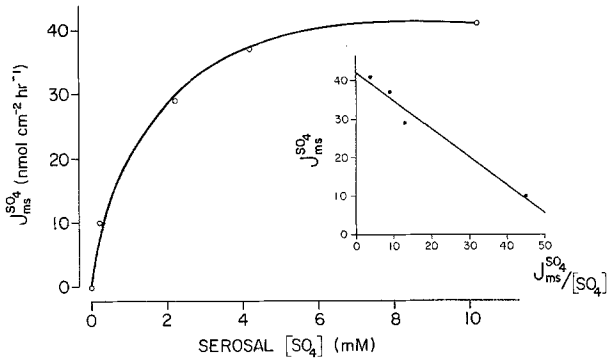

Fig. 1. *m*-to-*s* SO_4 flux as a function of serosal SO_4 concentration. The curve represents the mean flux measurements from four experiments. Tissues were bathed in gluconate Ringer's with 0.22 mM SO_4 in the mucosal medium and varying concentrations of SO_4 in the serosal medium. The data were normalized to the average value of $J_{ms}^{SO_4}$ measured when serosal SO_4 = 0, (15 nmol $cm^{-2}hr^{-1}$). In constructing the graph, this "basal flux", which is independent of serosal SO_4, was first subtracted from the measured flux values. The inset shows the Eadie-Hofstee plot of the data with the line drawn by linear regression analysis. (J_{max} = 42.2 and $K_{\frac{1}{2}}$ = 0.7)

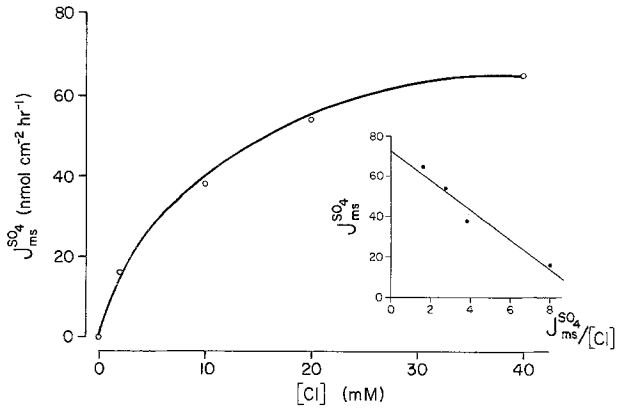

Fig. 2. *m*-to-*s* SO_4 flux as a function of Cl concentration. The curve represents the mean flux measurements from four experiments. Tissues were bathed in gluconate Ringer's containing various concentrations of Cl. SO_4 (0.22 mM) was present in the mucosal solution only. Data were normalized to the average value of $J^{SO_4}_{ms}$ measured when Cl=0 (15 nmol cm^{-2}hr^{-1}). This "basal flux" was subtracted from the measured flux values before construction of the graph. The inset shows the Eadie-Hofstee plot of the data with the line drawn by linear regression analysis. (J_{max}= 73.1 and $K_{\frac{1}{2}}$=7.4)

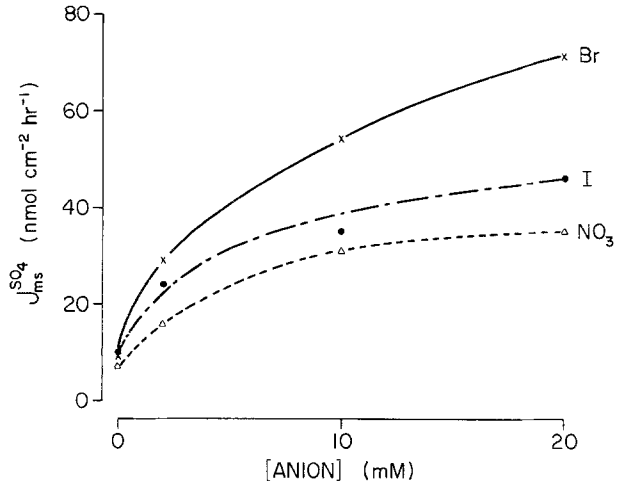

Fig. 3. *m*-to-*s* SO_4 flux as a function of Br (x), I (•) and NO_3 (△) concentration. Each curve represents the mean flux measurements from two experiments. Tissues were bathed in gluconate Ringer's containing various concentrations of the test anion. SO_4 (0.22 mM) was present in both mucosal and serosal solutions. Data were normalized to the averaged control value of $J^{SO_4}_{ms}$ measured when test anion=0 and serosal SO_4=0 (15 nmol cm^{-2}hr^{-1}). This "basal flux" was subtracted from the measured flux values before construction of the graph. The curves will be somewhat distorted at low concentrations of test anion by the presence of the small amount of SO_4 in the serosal solution; estimations of $K_{\frac{1}{2}}$ values will be similarly diminished in accuracy

exchange were both significantly higher than for SO_4/SO_4 exchange. The higher J_{max} for Cl was verified by measuring both Cl- and SO_4-stimulated *m*-to-*s* SO_4 fluxes on tissues from the same animals. Cl and serosal SO_4 concentrations were adjusted to 5–6 times their estimated $K_{\frac{1}{2}}$ values. In six paired experiments, the mean *m*-to-*s* SO_4 fluxes were 79.6±6.1 nmol cm^{-2} hr^{-1} at 40 mM Cl and 48.8±5.3 nmol cm^{-2} hr^{-1} at 4.22 mM SO_4 ($p<0.001$).

Table 2. Effect of HCO_3 and PO_4 on SO_4 fluxes in gluconate Ringer's

Conditions		$J^{SO_4}_{ms}$	$J^{SO_4}_{sm}$	$J^{SO_4}_{net}$
(A) Control	(3)	19.5±5.3	8.1±0.3	11.5±5.2
+HCO_3 25 mM (pH 7.4)		17.2±1.1	6.0±2.3	11.6±1.4
(B) Control	(2)	30.7	11.5	19.2
+PO_4 2.7 mM (pH 7.4)		28.9	11.7	17.2
(c) Control	(3)	14.5±2.5	–	–
+PO_4 4 mM (pH 7.2)		16.6±1.5	–	–

SO_4 fluxes are in nmol cm^{-2} hr^{-1}. Values are means alone or means ±1 SE for paired experiments on tissues from (*n*) animals. In (A) and (B), tissues were bathed in gluconate Ringer's containing 0.22 mM SO_4 at pH 7.4; HCO_3 and PO_4 substitutions were made in both mucosal and serosal solutions as indicated. In (C), the bathing solutions were at pH 7.2 and PO_4 mM was substituted on the serosal side only; also, SO_4 was omitted from the serosal solution to maximize the chance of seeing an effect of PO_4 on $J^{SO_4}_{ms}$. All solutions contained HEPES 3 mM: HCO_3 solutions were gassed with 5% CO_2 in O_2; all other solutions were gassed with 100% O_2. [*Note:* In (B), at pH 7.4, $[HPO_4^{2-}]$=2.1 mM and $[H_2PO_4^-]$=0.6 mM; in (C), at pH 7.2, $[HPO_4^{2-}]$=2.6 mM and $[H_2PO_4^-]$=1.3 mM (corrected for ionic strength)].

Figure 3 shows the stimulatory effects of Br, I and NO_3 on *m*-to-*s* SO_4 flux. The $K_{\frac{1}{2}}$ values for the three anions range from 3–6 mM. The J_{max} for Br is close to that for Cl, but those of I and NO_3 are smaller. A more quantitative comparison of the kinetic parameters is not possible since the number of experiments with Br, I and NO_3 was small and because the experimental conditions were not identical (*see* legend to Fig. 3). The stimulatory effects of all five of the above anions were completely blocked by 500 μM SITS, suggesting a common mode of action: in presence of near-maximally stimulating concentrations of each anion (≧20 mM), serosal SITS reduced the *m*-to-*s* SO_4 flux to less than 10 nmol cm^{-2} hr^{-1} (6.6–9.9).

In contrast to the effects of the above anions, HCO_3 (25 mM) and PO_4 (4 mM) did not stimulate *m*-to-*s* SO_4 flux (Table 2). Although the concentration of PO_4 employed were relatively low, equal concentrations of the anions shown in Figs. 1, 2, and 3 produced easily detectable changes. Nonetheless a comparatively low affinity of HPO_4 or H_2PO_4 for the exchange mechanism has not been excluded. Furthermore, since the ileum secretes HCO_3, pHs in the unstirred layer along the basolateral membrane may be lower than in the bulk medium; it is therefore possible that less than 25 mM HCO_3 was present at the basolateral surface of the epithelial cells. Thus a comparatively low affinity of HCO_3 for the exchange mechanism has not been excluded.

Table 3. Effect of serosal pH on SO_4 fluxes

Serosal pH	Cl-free Ringer's		2 mM Cl
	$J_{ms}^{SO_4}$	$J_{sm}^{SO_4}$	$J_{ms}^{SO_4}$
pH 7.4 (4)	26.4	12.0	35.7
pH 7.0 (2)	29.1	13.2	34.2
pH 7.8 (2)	27.2	14.5	39.8

SO_4 fluxes are in nmol cm^{-2} hr^{-1}. Values are means for paired experiments on tissues from (n) animals and are normalized to the mean control (pH 7.4) values as follows: $J_e^i(n) = (J_c/J_c^i) \times J_e^i$, where J_c = mean control flux for all experiments, J_e^i and J_c^i refer to experimental and control fluxes in a particular experiment i and $J_e^i(n)$ is the normalized experimental flux. Tissues were bathed in gluconate Ringer's with or without Cl (2 mM). SO_4 (0.22 mM) was present in both mucosal and serosal solutions. In experiments with 2 mM Cl, only m-to-s SO_4 fluxes were measured.

Effect of Serosal pH on SO_4 Fluxes

In order to examine the influence of pH on the SO_4 exchange mechanism, SO_4 fluxes were measured in gluconate Ringer's solutions at serosal pHs of 7.0, 7.4 and 7.8. Mucosal pH was kept at 7.4. Neither SO_4/SO_4 exchange nor SO_4/Cl exchange was altered by pH changes within this range (Table 3).

Effects of Inhibitors

Table 4 shows the effects on SO_4 fluxes of nine agents which are known to inhibit anion exchange in erythrocytes or Ehrlich ascites tumor (EAT) cells. Only SITS, DIDS and ANS were found to inhibit ileal SO_4 transport. Ethacrynic acid (1 mM) slightly inhibited the m-to-s SO_4 flux but it also slightly decreased the I_{sc} response to glucose, suggesting an effect on the Na gradient rather than on the serosal exchange mechanism. SITS proved far more potent than DIDS or ANS. Near-complete inhibition of SO_4 flux was obtained with 50 μM SITS whereas 50 μM DIDS was ineffective. It is worth noting that neither SITS, DIDS nor ANS inhibited the I_{sc} response to glucose, indicating no effect on the Na gradient at the concentrations employed.

One additional agent, NAP-taurine, which inhibits SO_4 fluxes in erythrocytes [4] and hepatocytes [7], was tested in gluconate Ringer's containing 0.22 mM SO_4 and 20 mM Cl. The experiment was performed in the dark since photolysis converts NAP-taurine to a highly reactive nitrene which acts as a general covalent labeling reagent. Neither m-to-s nor s-to-m SO_4 fluxes were altered by 100 and 500 μM NAP-taurine (*data not shown*).

The effects of SITS on SO_4 fluxes in gluconate Ringer's are shown in Table 1. These results should be compared with those for SITS in standard Ringer's (Table 4). While SITS inhibited the m-to-s flux in both Ringer's solutions, its effect on s-to-m flux differed in the two solutions: it produced minimal inhibition in standard Ringer's but reduced the s-to-m SO_4 flux by 80% in gluconate Ringer's. Thus the stimulation of the s-to-m flux observed upon replacement of Cl with gluconate was completely reversed by SITS.

Since SITS can block Cl-dependent SO_4 transport, it was of interest to determine its effect on Cl transport. The data in Table 5 indicate that SITS does not have a significant effect on Cl fluxes either in the presence or absence of SO_4.

Table 4. Effect of anion exchange inhibitors on SO_4 fluxes

Agent added	Cells in which inhibition demonstrated	$J_{ms}^{SO_4}$	$J_{sm}^{SO_4}$	J_{net}	$\Delta I_{sc}^{(Glu)}$
Control (30)	–	79.6	2.4	77.2	107
SITS 50 μM (4)	Erythrocytes [5]	13.1	2.2	10.9	102
SITS 500 μM (3)	EAT cells [22]	5.2	1.9	3.3	126
DIDS 50 μM (3)	Erythrocytes [5]	80.4	1.6	78.8	128
DIDS 500 μM (3)	EAT cells [23]	30.2	1.5	28.7	107
DNDS 500 μM (3)	Erythrocytes [1]	77.0	3.1	73.9	124
DADS 500 μM (2)	Erythrocytes [5]	80.1	3.4	76.7	111
ANS 500 μM (2)	Erythrocytes [12] EAT cells [24]	27.1	2.7	24.4	111
Phloretin (3) 500 μM	Erythrocytes [35] EAT cells [23]	102.9	2.5	100.4	109
Dipyridimole (2) 500 μM	Erythrocytes [9]	91.1	2.4	88.7	97
Furosemide (2) 1 mM	Erythrocytes [3]	92.8	2.5	90.3	113
Ethacrynic acid (3) 1 mM	Erythrocytes [27]	59.5	1.9	57.6	73

SO_4 fluxes are in nmol cm^{-2} hr^{-1} and ΔI_{sc} in μA. Values are means for paired experiments on tissues from (n) animals. J_{ms}, J_{sm} and ΔI_{sc} have been normalized to the mean control values, as described in the legend to Table 3. ΔI_{sc} = the change in I_{sc} following mucosal addition of glucose (to 10 mM) at the end of the flux period. *See* footnote 1 for full chemical names of the inhibitors. None of the agents tested caused a significant change in tissue conductance. (Experiments with stilbene derivatives were performed both in the light and in the dark since exposure of some of these molecules to light can cause the transisomeric form to convert to the cis form which could be less effective [12]; results of individual experiments indicated that light exposure did not alter the effectiveness of any of the four compounds used and data from the light and dark experiments have therefore been pooled).

Discussion

The present study demonstrates that the m-to-s SO_4 flux in ileal epithelium is stimulated by Cl, Br, I, NO_3 and serosal SO_4. For all five anions the effect is saturable and can be blocked by serosal SITS. These

Table 5. Effects of SITS on Cl fluxes

Ringer's solution		J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}^{Cl}
$^1/_2$ – Chloride Ringer's				
Cl = 65 mM	Control (5)	8.6±0.8	3.4±0.2	5.3±0.7
SO_4 = 31 mM	+SITS (5)	8.5±0.9	3.8±0.4	4.5±1.0
Gluconate Ringer's				
Cl = 20 mM	Control (2)	3.0	1.5	1.5
SO_4 = 0	+SITS (2)	3.0	1.0	2.0

Cl fluxes are in μmol cm^{-2} hr^{-1}. Values are means alone or means ± 1 SE for paired experiments on tissues from (*n*) animals. SITS (500 μM) was added to the serosal medium. Epinephrine (50 μM) was added to the serosal solution to stimulate Cl absorption [11] and maximize the chance of detecting an inhibitory effect of SITS.

Table 6. Comparison of effects of anion transport inhibitors in ileum, EAT cells and erythrocytes

Inhibitor	Ileum		EAT Cell [22, 23, 33]		Erythrocyte [3, 5, 6, 12, 35]	
	SO_4	Cl	SO_4	Cl	SO_4	Cl
SITS	+	−	+	−	+	+
DIDS	+	?	+[a]	−[a]	+	+
ANS	+	?	+	−	+	+
Furosemide	−	?	−	+	+[b]	+
Phloretin	−	?	+	−	+	+

[a] Refers to effect of irreversibly bound H_2DIDS.
[b] Personal communication from Dr. R.B. Gunn and Mark Milanick.
Key: + indicates inhibition,
− indicates no effect detected
? indicates no data available.

results together with those in the preceding paper [30] indicate that there is a carrier-mediated process located at the basolateral membrane which transports SO_4 out of the cell in exchange for the inward transport of one or more anions from the serosal medium. Neither transepithelial [30] nor trans-brush border [26] SO_4 transport (the latter measured in vesicles) appears to be electrogenic. Therefore trans-serosal border SO_4 transport is probably also electroneutral. In an exchange of divalent SO_4 for monovalent Cl, electroneutrality could be achieved by a stoichiometry of transport of 1 SO_4:2 Cl. Alternatively, one SO_4 plus one proton could exchange for one Cl, as in the red blood cell [19]. Trans-ileal SO_4 fluxes were not altered when the serosal pH was varied in the range 7.0–7.8. This argues against SO_4-proton co-transport but does not exclude it, since the proton concentration may not be rate-limiting in this pH range. A further possibility, not investigated here, is that SO_4 is accompanied by K^+ or Na^+ as suggested for the electroneutral one for one SO_4/Cl exchange of EAT cells [33].

Ileal SO_4 transport is inhibited by SITS, DIDS and ANS, but not by several other inhibitors of anion permeability in erythrocytes and other cell types. SITS is by far the most potent inhibitor; by contrast, in red blood cells DIDS is more potent than SITS [5]. It is not readily apparent from a comparison of the chemical structures of all agents tested why only these three particular compounds should inhibit SO_4 transport, or why SITS should be so much more potent than DIDS or ANS. For a series of inhibitors in erythrocytes, no correlation was found between inhibitory potency and reversibility of membrane binding [5]. A similar lack of correlation was noted in the present study, assuming reversibility of binding in ileal epithelial cells to be similar to that in erythrocytes and EAT cells. ANS, a reversibly-binding compound, was as effective as DIDS, a covalently binding compound, and SITS, which exhibits mixed binding, was more potent than either DIDS or ANS. It is of interest that 50 μM DIDS is without effect at 0.22 mM SO_4 (present study) but is inhibitory at 2.35 mM SO_4 (*see* preceding paper [30]). It is possible that the SO_4/Cl concentration ratio modifies sensitivity to DIDS and perhaps to other inhibitory agents.

Table 6 compares the effects of five inhibitors of anion permeability on SO_4 and Cl fluxes in ileum, EAT cells and red blood cells. In the red blood cell, Cl and SO_4 fluxes are inhibited by the same agents: this is in accord with the titratable carrier model for anion transport [15] whereby Cl and SO_4 are transported via the same anion exchanger. In EAT cells, however, a different pattern emerges which resembles more closely that of the ileal epithelial cell. In EAT cells there appear to be two separate pathways for Cl uptake [33]: one involves exchange for intracellular SO_4 and is blocked by SITS; the second is a quantitatively more important SITS-insensitive pathway which is inhibited by furosemide. SITS alone had no measurable effect on Cl uptake, but when the major Cl pathway was blocked with furosemide, an inhibitory effect of SITS on the remaining Cl transport was revealed. In the present study (*see* Table 5), Cl absorption was not significantly altered by SITS, despite the existence in the basolateral membrane of a SITS-sensitive, SO_4-coupled Cl flux. Data in the preceding paper [30] suggest that the transcellular portion of J_{ms}^{SO} to be expected at 31 mM SO_4 (i.e., J_{max}^{SO}) is about 1.0 μmol cm^{-2} hr^{-1}. In principle, this SO_4 flux could contribute as much as 2.0 μmol cm^{-2} hr^{-1} to J_{sm}^{Cl}. SITS inhibition of this flux should have been easily detected. The stoichiometry of SO_4/Cl exchange could be 1:1 rather than 1:2, however (i.e., KSO_4

or HSO_4/Cl exchange), but a 1.0 μmol change should still have been detectable. Therefore, much of the Cl entering the ileal cell in exchange for SO_4 is probably recycled to the serosal medium by another pathway. It is also worth noting that SITS did not significantly alter Cl fluxes in the absence of SO_4 (Table 5), indicating that a SO_4-independent effect of SITS on Cl transport was not obscured by an oppositely directed effect on a SO_4-dependent Cl flux.

In standard Cl Ringer's containing 0.22 mM SO_4, the *s*-to-*m* flux of SO_4 was not significantly reduced by SITS (*see* Table 4), suggesting that almost all of this flux represents a simple diffusional, probably paracellular flow. In gluconate Ringer's (Cl-, HCO_3- and PO_4-free) the *m*-to-*s* flux of SO_4 is reduced by 70% whilst the *s*-to-*m* flux is increased by 400%. Both of these results are consistent with anion exchange: removal from the serosal medium of anions which may readily exchange for cell SO_4 reduces the rate of exit of SO_4 at the basolateral membrane and therefore decreases the *m*-to-*s* flux; on the other hand, in the absence of competing anions (such as Cl), the small amount of SO_4 present in the serosal medium can more readily exchange for cell SO_4 producing a marked increase in *s*-to-*m* flux. This increase is completely reversed by SITS, which is further evidence that it is due to a higher rate of SO_4/SO_4 exchange. The remaining SITS-insensitive flux (2.2 nmol cm^{-2} hr^{-1}) is of similar magnitude to the SITS-insensitive flux in standard Ringer's and again probably corresponds to a paracellular flow. In the preceding paper, at a 10-fold higher SO_4 concentration, 75% of the *s*-to-*m* flux (in standard Ringer's) was inhibited by SITS. At this higher ratio of SO_4 to Cl concentrations, a larger proportion of the *s*-t-*m* flux would be transcellular and SITS-inhibitable. Using the *s*-to-*m* flux values obtained at 2.35 mM SO_4 (*see* preceding paper [30]) the calculated permeability of the SITS-insensitive diffusional pathway is 7.7×10^{-3} cm hr^{-1}, a value not significantly different from that obtained in the present work (1.9 from Table 4/ 0.22 mM $= 8.6 \times 10^{-3}$ cm hr^{-1}).

In gluconate Ringer's there remains a small but significant net flux of SO_4. Since SO_4/SO_4 self-exchange would not result in a net flux, $J_{net}^{SO_4}$ under these circumstances could represent one of three processes: (*i*) SO_4/gluconate exchange; (*ii*) a net SO_4 efflux via the anion exchange carrier without counterflux of an anion from the serosal medium ("slippage"); or (*iii*) another transport pahtway for SO_4. This last possibility is unlikely since serosal SITS nearly abolished $J_{net}^{SO_4}$.

SO_4/Cl exchange occurs at a faster rate than SO_4/SO_4 self-exchange, as indicated by the higher J_{max} for the Cl-stimulated SO_4 fluxes. The calculated $K_{\frac{1}{2}}$ value for serosal SO_4 (0.7 mM) is 10-fold lower, however, than that for Cl (7.4 mM): assuming that the anion translocation stage of the exchange process is slow relative to the substrate-membrane binding stage, this suggests that the affinity of the exchange system for SO_4 is greater than for Cl. In terms of a diffusable carrier model, it appears therefore that the molecular features of the substrate which determine the rate of movement of the carrier-anion complex from one side of the membrane to the other are different from those which affect binding to the substrate site. Qualitatively similar findings have been reported for erythrocyte anion exchange: SO_4 is transported at a very much slower rate than Cl, but has a higher affinity for the substrate binding site [8, 29].

In red blood cells a remarkably large range of substrates can be transported by the anion exchange system [20]. The principal physiological substrates are Cl and HCO_3, and Cl/HCO_3 exchange (the Hamburger shift [16], plays a central role in control of blood pCO_2. In the ileal epithelial cell Cl, Br, I, NO_3 and SO_4 can all act as substrates for the anion exchange system at the basolateral membrane. HCO_3 and PO_4 however do not appear to interact with this system, although a low affinity for the exchange mechanism has not been excluded for either anion. This result is somewhat surprising, particularly with respect to HCO_3 which has been implicated in anion exchange processes in a number of epithelial tissues. (For example: *Amphiuma* small intestine [34]; rat ileum [17]; human ileum [31]; turtle bladder [18, 21]). Ullrich and coworkers [32] describe a HCO_3-dependence of SO_4 reabsorption in the proximal convoluted tubule of rat kidney. However, removal of HCO_3 produced only a modest decline in SO_4 transport and it is not clear whether this represents a metabolic interdependence or a more specific association between HCO_3 and SO_4 transports.

Several recent studies on the mammalian kidney have revealed similarities between SO_4 transport processes in this tissue and those in rabbit ileum. Active absorption of SO_4 has been demonstrated in the proximal convoluted tubule of rat kidney [32]. Uptake across renal cortical brush border membranes is a Na-dependent process [25, 28]. Grinstein et al. [14] found that SO_4 efflux from renal cortical basolateral membrane vesicles is inhibited by 50 μM DIDS: no similar inhibitory effect of DIDS was found on SO_4 efflux from brush border membrane vesicles. The recent work of Brazy and Dennis [2] on rabbit proximal convoluted tubule suggests that SO_4 transport involves a SITS-inhibitable anion exchange mecha-

nism located at the basolateral membrane. Detailed information on the interaction of other anions with the exchange mechanism is not yet available, but PO_4 was found to have no effect on SO_4 transport.

In summary, the results of the present study indicate that SO_4 transport across the basolateral membrane of the rabbit ileal mucosa has features which satisfy a number of criteria for carrier-mediated anion exchange. It exhibits (*i*) susceptibility to specific modifying agents, such as SITS; (*ii*) trans-stimulation of the *m*-to-*s* flux by Cl and other anions; (*iii*) saturation kinetics; and (*iv*) competition for *s*-to-*m* transfer among anions placed on the serosal side. SO_4 entry across the brush border membrane is coupled to Na entry and therefore utilizes the Na gradient as an energy source. SO_4 exits at the basolateral border by exchange for serosal Cl. The chemical gradient for Cl may be a second energy source for transepithelial SO_4 transport. The stoichiometry of the exchange process and the form(s) of SO_4 as it crosses the basolateral membrane (e.g., SO_4^{2-}, HSO_4^-, KSO_4^-, etc.) remain to be determined.

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