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Active Sulfate Absorption in Rabbit Ileum: Dependence on Sodium and Chloride and Effects of Agents that Alter Chloride Transport

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Summary. Unidirectional fluxes of $^{35}SO_4$ across and into rabbit ileal epithelium were measured under short-circuit conditions, mostly at a medium SO_4 concentration of 2.4 mM. Unidirectional mucosa (*m*)-to-serosa (*s*) and *s*-to-*m* fluxes (J_{ms}, J_{sm}) were 0.456 and 0.067 μmoles hr^{-1} cm^{-2}, respectively. J_{ms} was 2.7 times higher in distal ileum than in mid-jejunum. Ouabain abolished net SO_4 transport (J_{net}) by reducing J_{ms}. Epinephrine, a stimulus of Cl absorption, had no effect on SO_4 fluxes. Theophylline, a stimulus of Cl secretion, reduced J_{ms} without affecting J_{sm}, causing a 33% reduction in J_{net}. Other secretory stimuli (8-Br-cAMP, heat-stable enterotoxin, Ca-ionophore A23187) had similar effects. Replacement of all Cl with gluconate markedly reduced J_{net} through both a decrease in J_{ms} and an increase in J_{sm}. The anion-exchange inhibitor, 4-acetoamido-4′-isothiocyano-2,2′-sulfonic acid stilbene (SITS), when added to the serosal side, reduced J_{ms} by 94%, nearly abolishing J_{net}. SITS also decreased J_{sm} by 75%. Mucosal SITS (50 μM) was ineffective. 4,4′-diisothiocyano-2,2′-sulfonic acid stilbene (DIDS) had effects similar to SITS but was less potent. Measurements of initial rates of epithelial uptake from the luminal side (J_{me}) revealed the following: (1) J_{me} is a saturable function of medium concentration with a V_{max} of 0.94 μmoles hr^{-1} cm^{-2} and a $K_{\frac{1}{2}}$ of 1.3 mM; (2) replacing all Na with choline abolished J_{me}; (3) replacing all Cl with gluconate increased J_{me} by 40%; (4) serosal SITS had no effect on J_{me}; and (5) stimuli of Cl secretion had no effect on J_{me} or increased it slightly. Determination of cell SO_4 with $^{35}SO_4$ indicated that, at steady-state, the average mucosal concentration is 1.1 mmoles per liter cell water, less than half the medium concentration. Cell SO_4 was increased to 3.0 mM by adding SITS to the serosal side. Despite net transport rates greater than 1.4 μEq hr^{-1} cm^{-2}, neither addition of SO_4 to the SO_4-free medium nor addition of SITS to SO_4-containing medium altered short-circuit current. The results suggest that (1) ileal SO_4 absorption consists of Na-coupled influx (symport) across the brush border and Cl-coupled efflux (antiport) across the basolateral membrane; (2) the overall process is electrically neutral; (3) the medium-to-cell Cl concentration difference may provide part of the driving force for net SO_4 absorption; and (4) since agents affecting Cl fluxes (both absorptive and secretory) have little effect on SO_4 fluxes, the mechanisms for their transcellular transports are under separate regulation.

Key words: rabbit ileum; sulfate transport; sodium dependence; chloride dependence

Cl is both actively absorbed and secreted in the small intestine. At a molecular level the processes involved are poorly understood. In a purely physiological sense, however, several of their essential features have become apparent [12]. The uphill step in active Cl absorption appears to be Na−Cl cotransport across the brush border membrane; Cl then exits across the basolateral membrane by a nonconductive pathway (possibly KCl cotransport) [7, 28, 33]. Analogously, the uphill step in active Cl secretion appears to be Na−Cl cotransport across the basolateral membrane; Cl then exits across the luminal membrane, probably by a conductive pathway [2, 12, 19, 24, 31, 36]. Because both processes couple Na and Cl movements, both are energized, at least in part, by the Na gradient. Both processes are also regulated by agents that alter intracellular concentrations of cyclic nucleotides and Ca [3, 8, 10, 12, 14, 16, 25, 32]. More specifically, such agents have been shown

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to inhibit Na – Cl cotransport across the brush border [16, 25], thereby inhibiting net Cl absorption and, based on studies of other Cl-secreting vertebrate epithelia [12, 19, 24, 31, 36], they also appear to increase the Cl conductance of the luminal membrane, thereby lifting the barrier to net secretion.

The anionic specificities of these transport processes have thus far received little attention. In order to gain a broader understanding of how the intestine handles small water-soluble anions, we have investigated ileal SO_4 transport. Prior studies have shown that SO_4 is actively absorbed there by a Na-dependent mechanism [1, 5, 23, 29]. In addition to examining the general features of this system, we have tested the effects on SO_4 fluxes of agents known to alter intestinal Cl transport.

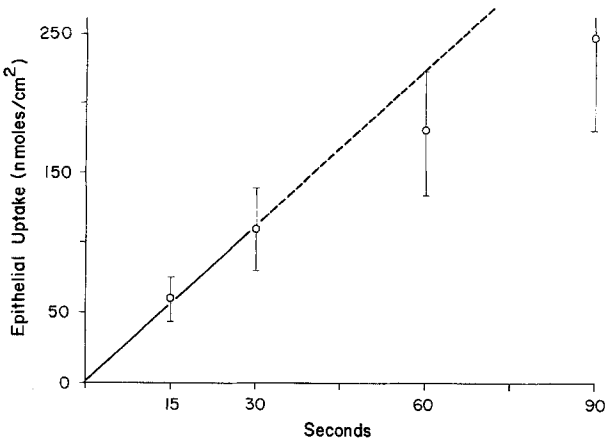

Fig. 1. Tissue uptake of $^{35}SO_4$ as a function of time. $^{35}SO_4$ and [^{3}H]-PEG added to the luminal side at time zero. Uptakes shown have been corrected for ^{35}S present in the extracellular space (^3H space). Medium SO_4 concentration = 2.35 mM. Values are means ± SE for 8 determinations at each point in time

Materials and Methods

Materials

N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid (HEPES), 8-Br-cAMP, ouabain, theophylline, sodium gluconate, choline chloride and choline bicarbonate were obtained from Sigma (St. Louis, Mo.). 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS) and 4,4′-diisothiocyano-stilbene-2,2′-disulfonate (DIDS) were obtained from Pierce (Rockford, Ill.). Partially purified heat-stable *Escherichia coli* enterotoxin (ST), prepared as previously described [27], was a gift from W.A. Laird (National Bureau of Standards, Washington, D.C.).

[^{3}H]-PEG (mol wt 900, 5 mCi/mmol) and Na_2 $^{35}SO_4$ (113 mCi/mol) were obtained from New England Nuclear (Boston, Mass.). Ionophore A23187 was a gift from Dr. Robert Hamill (Lily Research Labs, Indianapolis, Ind.).

Experimental Preparations

Rabbit ileal or jejunal mucosa was obtained from New Zealand white, male rabbits (2–3 kg) which had been fed a standard rabbit chow and water *ad lib*. Rabbits were killed by cervical dislocation. Distal ileum or jejunum, taken 24–36 inches from pylorus, was quickly excised, opened along its mesenteric border and rinsed with ice-cold standard Ringer's (see below). All tissues were kept at ice bath temperature and gassed with 5% CO_2 in O_2 prior to use. The two major muscle layers were removed as previously described [17]. Unless otherwise stated, tissues were incubated in a standard Ringer's (in mmol/liter. Na, 141; K, 5; Ca, 1.25; Mg, 1.1; Cl, 117.5; HCO_3, 25; H_2PO_4, 0.35; HPO_4, 1.65; SO_4, 2.35) continuously bubbled with 5% CO_2 in O_2. The following modified Ringer's solutions were also employed:

(1) *SO_4-free Ringer's:* Standard Ringer's with 2.35 mM SO_4 replaced by Cl.

(2) *Na-free Ringer's:* Standard Ringer's with all Na replaced by choline.

(3) *Cl-free Ringer's:* Standard Ringer's with all Cl replaced by gluconate.

(4) *Cl and HCO_3-free Ringer's:* Standard Ringer's with all Cl and HCO_3 replaced by gluconate; 10 mM HEPES, titrated to pH 7.4 with 1 N NaOH, added as buffer; solution bubbled with 100% O_2.

(5) *Variable SO_4-Ringer's:* Solutions with different SO_4 concentrations (0.125 to 10 mM) made from SO_4-free Ringer's by replacing Cl with equivalent amounts of SO_4 and adding mannitol to keep osmolarity constant.

In all experiments, glucose (10 μmol/ml) was added to the serosal bathing medium and an equimolar amount of mannitol to the mucosal medium.

Transmural SO_4-Flux Measurements

Sections of intestinal mucosa (usually 6) were mounted in Ussing chambers and their transepithelial electric potential differences (PD), conductances (G_t) and short-circuit currents (I_{sc}) were determined as previously described [19]. Thirty to 45 min after tissues were mounted in chambers, Na_2 $^{35}SO_4$ (0.5–1 μCi) was added to one side and unidirectional transmural fluxes from mucosa (*m*)-to-serosa (*s*) and from *s*-to-*m* were determined under short-circuit conditions as previously described [11] from initial samples taken 20 min after the addition of radioisotope and duplicate final samples taken 30–34 min thereafter. In preliminary experiments, it was established that steady-state rates of $^{35}SO_4$ transfer from *s*-to-*m* and from *m*-to-*s* were attained within 15 min of introducing isotopes (*data not shown*).

SO_4 Influx Measurements

Sections of mucosa were mounted mucosa up in multiport "influx" chambers similar to those described previously [14]. Ringer's solutions were circulated by gas lift and maintained at 37 °C in a constant temperature hood. Twenty to 30 min after mounting tissues, 1 μCi Na_2 $^{35}SO_4$ and 4 μCi of [^{3}H]-PEG were added on the mucosal side (vol = 2 ml). At variable times thereafter (usually 30 sec) the mucosal solution was changed and tissues were punched out and their radioactivity was extracted and assayed as previously described [14]. Wet weights were obtained prior to extraction. Although fluxes were expressed per cm^2 surface area, in each experiment area was first normalized by wet wt to an average value of mg wet wt/cm^2. SO_4 influxes were corrected for extracellular contamination by subtracting the [^{3}H]-PEG "space". Influxes were measured under short-circuit conditions. The time course of $^{35}SO_4$ uptake (2.35 mM SO_4) is shown in Fig. 1. Since uptake was linear for at least 30 sec, this interval was used for all subsequent influx measurements.

Table 1. SO_4 fluxes across ileal and jejunal mucosa

Conditions	J_{ms}	J_{sm}	J_{net}	I_{sc}	G_t
A. Effect of Time (6)					
control (40–70 min)	480±31	64± 4	416±33	1.7±0.34	25±1.3
control (90–120 min)	408±25[a]	63± 5	344±26[a]	2.0±0.44	26±1.8
B. Effect of ouabain (3)					
control	411±80	68± 8	344±81	1.1±0.87	31±3.8
ouabain (0.25 mM)	97±19[b]	80± 7	17±12[b]	0.2±0.12	21±1.3
C. Ileum vs. jejunum (3)					
ileum	438±16	–	–	1.6±0.97	33±4.6
jejunum	163±13[a]	–	–	0.8±0.41	25±1.2
D. Effect of epinephrine (3)					
control	412±17	61±10	351±10	2.0±0.48	24±3.4
epinephrine (0.1 mM)	422±62	57± 8	364±72	−0.4±0.51[b]	30±1.0
E. Effects of secretory stimuli (E1=5; E2=4)					
1. control	400±29	60± 5	340±32	1.9±0.49	25±1.8
theophylline	293±36[a]	64± 7	228±34[a]	4.3±0.18[a]	22±1.2
2. control	555±39	–	–	−0.3±0.67	28±2.4
8-Br-cAMP (0.5 mM)	308±31[b]	–	–	3.9±0.58[b]	22±2.5
St (12 mouse U/ml)	437±33[b]	–	–	2.2±0.21[b]	25±0.5
A23187 (1 μM)	399±15[b]	–	–	1.9±0.21[b]	23±1.1

Means ±1 SE for (n) animals. Unless otherwise stated, all experiments were done on ileal mucosa. Fluxes are in nmol hr^{-1} cm^{-2}, I_{sc} in μEq hr^{-1} cm^{-2} and G_t in mmho cm^{-2}. All agents except for heat-stable enterotoxin (ST) were added to the serosal side and ST was added on the mucosal side. Fluxes were measured beginning 30–45 min after adding ouabain and beginning 15–30 min after other additions.
[a] $p<0.01$. [b] $p<0.05$.

Measurement of Intracellular SO_4 Concentrations

Tissues were mounted and incubated in influx chambers as described above. After a 20–30 min pre-equilibration period, $^{35}SO_4$ (0.7 μCi/ml) and [^{3}H]-PEG (1.0 μCi/ml) were added to both sides. Mucosal and serosal solutions were mixed by repeated cross-transfers to assure equal radioisotope concentrations on both sides. After 45 min, tissues were removed, blotted and weighed. They were then extracted overnight in 5% trichloroacetic acid containing 5 mM Na_2SO_4. ^{3}H and ^{35}S in the extracts were then assayed by liquid scintillation spectrometry. Extracellular water, as measured with [^{3}H]-PEG, was on the average about 25% of tissue net wt. Cell water was assumed to be 85% of the difference between tissue net wt and extracellular water.

Statistics

The significance of differences between mean values was analyzed using Student's t-test for paired variates. Results are generally shown as means ±1 SE. For transmural fluxes, the degrees of freedom are one less than the number of animals; in the influx experiments, the degrees of freedom are one less than the number of pairs of ports (4 pairs per animal). The order of pairing was determined prior to beginning each experiment with alternate ports serving as controls.

Results

SO_4 Fluxes Across Ileum and Jejunum and Effects of Agents that Modify NaCl Transport

Unidirectional and net SO_4 fluxes (2.35 mM SO_4 on both sides) are shown in Table 1. Under control conditions, the m-to-s flux varied from 0.4 to 0.55 and the s-to-m flux from 0.06 to 0.07 μmol hr^{-1} cm^{-2}. The m-to-s flux decreased by 15% from the first to the second hour *in vitro* (Table 1 *A*). Ouabain markedly decreased J_{ms}, essentially abolishing the net flux (Table 1 *B*). This suggests involvement of the Na gradient in net SO_4 transport and is consistent with prior findings of Na-dependence [1, 5, 23, 29]. Since others have found that SO_4 absorption is greater in ileum than in jejunum or duodenum [1, 23], we compared J_{ms} in ileal and jejunal mucosa from the same rabbits (Table 1 *C*). The jejunal J_{ms} for SO_4 was 37% of the ileal J_{ms}. In contrast, I_{sc} responses to the mucosal-side addition of D-glucose, which reflect Na-glucose cotransport, were similar in both segments[1]; differences between jejunal and ileal SO_4 fluxes cannot therefore be attributed to variations in Na gradient.

Table 1 also shows the effects of several agents that either enhance or inhibit Cl absorption. Epinephrine, through α-adrenergic receptors, stimulates Na and Cl absorption and inhibits HCO_3 secretion [6, 9]. It had no effect on SO_4 fluxes (Table 1 *D*). Theophylline [8, 25], 8-Br-cAMP [32], heat-stable enterotoxin [10, 16] and Ca ionophore A21387 [3] all inhibit

[1] Addition of 10 μmoles/ml of D-glucose to the mucosal side increased I_{sc} by 1.5±0.16 μEq hr^{-1} cm^{-2} in ileum and 1.9±0.07 in jejunum (three experiments).

Table 2. Effects on SO_4 fluxes of replacing Cl and HCO_3 with gluconate

Experiment number	$[HCO_3]$	J_{ms}		J_{sm}		J_{net}		I_{sc}	
		Control	Cl-free	Control	Cl-free	Control	Cl-free	Control	Cl-free
1.	25 mM	363	259	63	176	300	83	1.1	2.1
2.	25 mM	493	233	53	178	440	55	0.3	2.1
3.	0	398	194	96	165	302	29	2.0	1.6
4.	0	316	207	79	149	237	58	1.5	1.6
5.	0	444	231	93	185	351	46	2.6	1.6
Pooled mean ±1 SE		403±35	225±13	77±10	171±7	326±37	54±10	1.5±0.4	1.8±0.1

SO_4 fluxes are in nmol hr^{-1} cm^{-2}; I_{sc} in μEq hr^{-1} cm^{-2}. Pooled results for Cl-free and Cl, HCO_3-free experiments indicate significant changes from all control SO_4 fluxes; $p<0.01$.

Table 3. Effects of SITS and DIDS on SO_4 fluxes

Addition	J_{ms}	J_{sm}
A. To Serosal Side		
control	536±63 (7)	72±4 (3)
SITS (0.5 mM)	30± 7 (4)	18±4 (3)
SITS (50 μM)	60±10 (7)	–
DIDS (0.5 mM)	113 (n=2; 147, 79)	28 (n=2; 22, 34)
DIDS (50 μM)	285±83 (3)	80±6 (3)
B. To Mucosal Side		
control	372±51 (3)	–
SITS (50 μM)	342±51 (3)	–

Means ±1 SE for (n) animals,or means and individual results when n=2. Fluxes are in nmol hr^{-1} cm^{-2}. SITS and DIDS were added 30–45 min before the flux period

NaCl absorption and stimulate Cl secretion. They inhibited $J_{ms}^{SO_4}$ by 20 to 45%, the largest effect being seen with 8-Br-cAMP.

Cl-Dependence of SO_4 Transport and Effects of Substituted Stilbenes

Replacement of Cl with gluconate markedly inhibited net SO_4 absorption (Table 2). This was the result of both a decrease in J_{ms} and an increase in J_{sm}. Replacement of HCO_3 as well as Cl gluconate did not have a significant additional effect.

Since the above experiments suggested $Cl-SO_4$ exchange as an essential step in transileal SO_4 absorption, the effects of SITS and DIDS, both of which inhibit anion exchange in erythrocytes [4], were examined (Table 3). SITS (0.5 mM), added on the serosal side, markedly reduced both J_{ms} and J_{sm} and almost completely abolished J_{net}. SITS at 50 μM was only slightly less effective. In contrast, 50 μM SITS added to the luminal side had no effect on J_{ms}. DIDS had the same effects as SITS, although it was less potent.

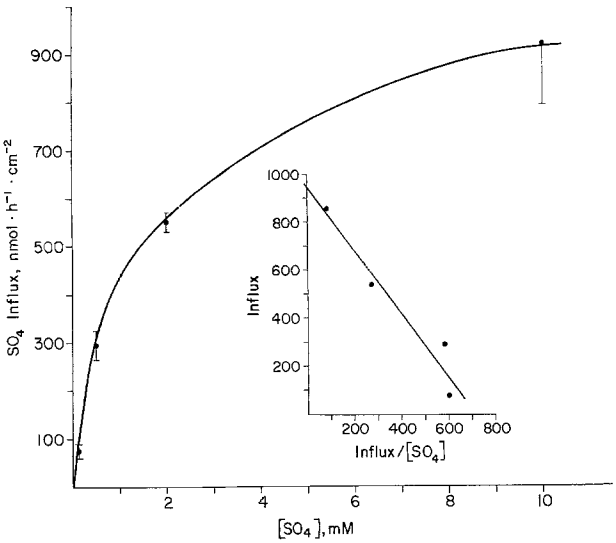

Fig. 2. Concentration-dependence of SO_4 influx. SO_4 concentration was varied by replacing Cl on a 2 for 1 molar basis. Mannitol was also added to keep osmolarity constant. Values are means ±SE of three or four determinations on tissues from three animals. For each animal, four tissues were exposed to 2.0 mM SO_4 and four tissues to one of the other concentrations. Influxes at 2 mM SO_4 differed among the three animals by less than 4% from the group mean. The insert shows an Eadie-Hofstee linear transformation of the cellular influxes. These were calculated as total influx minus paracellular flux, the latter being equal to the SITS-insensitive J_{sm} from Table 3 divided by 2.35 and multiplied by the SO_4 concentration tested. The correction was negligible at all concentrations except 10 mM, at which it still represents only 7.3% of the total influx. The insert suggests a linear relation (line drawn by simple regression analysis) with a J_{max} of 944 nmol hr^{-1} cm^{-2} and a $K_{\frac{1}{2}}$ of 1.3 mM

SO_4 Influx Across the Brush-Border

The features of SO_4 transport across the brush-border membrane were directly examined by determining SO_4 influx (J_{me}) from mucosal medium into the epithelium. As shown in Fig. 2, SO_4 influx is a first-order saturable function of medium concentration with a $K_{\frac{1}{2}}$ of 1.3 mM and a maximal influx of nearly 1.0 μmol hr^{-1} cm^{-2}. The $K_{\frac{1}{2}}$ is higher than that ob-

Table 4. SO_4 influxes across brush border

Experimental condition	Influxes (nmol hr^{-1} cm^{-2})	
	Control	Experimental
A. Effects of ion substitutions and SITS		
Na-free (7,2)	606 ± 69	−5 ± 11[a]
Cl-free (8,2)	448 ± 45	621 ± 64[a]
SITS, 0.5 mM (8,2)	296 ± 56	256 ± 38
B. Effects of stimuli of Cl secretion		
Theophylline, 5 mM (13,7)	377 ± 50	475 ± 48[a]
8-Br-cAMP, 0.5 mM (4,2)	544 ± 126	511 ± 107
ST, 15 mouse U/ml (6,3)	475 ± 92	572 ± 132
A23187, 1 μM (5,3)	458 ± 82	508 ± 50
Secretagogue in Na-free medium (3,2)	−24 ± 7.9	11 ± 16

Means ±1 SE for (m, n) experiments, where m = number of ports and n = number of animals. Control fluxes were measured in Na-free Ringer's. In Na-free experiments, 10 mM Na was present on the serosal side and none on the mucosal side. All agents were added 15–30 min prior to influx measurements. ST was added to the luminal side and other agents to the serosal side. Secretagogues employed for the 3 Na-free experiments were 8-Br-cGMP in one and combined addition of theophylline and A23187 in two. For the Na-free experiments in the top row, results with and without secretagogues were pooled.

[a] Different from control, $p < 0.05$. (Statistical comparison based on paired ports, i.e., "m".)

served by Lücke et al. [23] in rat ileal brush-border vesicles (about 0.3 mM). This difference could be a species variation or an unstirred layer effect [37]. It could also be due to the fact that their vesicles initially contained no SO_4. Substantial trans-stimulation was demonstrated in their study and would be a factor in the present experiments since the tissues were preincubated with SO_4 for 30–40 min prior to measuring influx.

Table 4 shows effects on J_{me} of Na and Cl replacements and of SITS and theophylline. Replacement of Na with choline abolished SO_4 influx. Replacement of Cl with gluconate increased J_{me} by 40%, suggesting modest competition between Cl and SO_4 for the SO_4 transporter. Addition of 0.5 mM SITS to the serosal medium had no effect on J_{me}, providing further evidence that the inhibitory effect of SITS on transmural fluxes is confined to a site on the basolateral membrane.

Theophylline and other secretory stimuli inhibit NaCl cotransport across the ileal brush border [16, 25]. Since theophylline partially inhibits transmural SO_4 absorption, it was of interest to determine whether or not his inhibitory action is exerted on the brush-border transporter, as is the case with Cl. Theophylline not only failed to inhibit J_{me} but actually increased it by 25%. In order to ascertain that this result was not due to the appearance of a Na-independent SO_4 influx, the effect of theophylline was also determined in Na-free Ringer's: there was no detectable influx in the absence of Na.

Intracellular SO_4 Concentration in the Presence and Absence of SITS

Intracellular exchangeable SO_4 was determined by equilibrating tissues with $^{35}SO_4$ and an extracellular marker (^{3}H-PEG) for 45 min. These experiments were performed on tissues mounted in the influx chambers and exposed on both sides to identical concentrations of radioisotopes. The results of four paired experiments on tissues from two rabbits were as follows: under control conditions (standard Ringer's), intracellular SO_4 was 1.1 ± 0.05 mmoles per liter cell water; this value was increased to 3.0 ± 0.35 mM by addition of SITS (0.1 mM) to the serosal side.

Nonelectrogenicity of SO_4 Absorption

Following preincubation in SO_4-free standard Ringer's, addition of SO_4 to both sides (6 μmol/ml replacing 12 μmol/ml of Cl) had no effect on I_{sc} (two experiments). If SO_4 were absorbed in the absence of any obligatory coupling to another ion flux, then about 1.5 μEq hr^{-1} cm^{-2} (or 40 μA/cm^2) of charge transfer would be expected; one-fourth or less of the resulting decrease in I_{sc} would have been readily detected. Similarly, complete inhibition of SO_4 absorption by addition of SITS had no effect on I_{sc}. We conclude therefore that ileal SO_4 absorption is electrically neutral or very nearly so.

Discussion

This study describes the principal features of a system for active SO_4 absorption present in rabbit ileum and, to a lesser extent, in jejunum. The system is electrically neutral and appears to have two components: (1) Na−SO_4 cotransport across the brush-border, and (2) SO_4−Cl exchange across the basolateral membrane.

Na-Dependent SO_4 Influx Across the Brush-Border

Three observations attest to the presence of a Na−SO_4 cotransporter in the brush border: (1) influx is saturable and Na-dependent; indeed, no influx could be detected in the absence of Na; (2) ouabain abolishes net absorption; and (3) SO_4 accumulates intracellularly above electrochemical equilibrium (enterocytes are about 40 mV electronegative relative to normal bathing medium [30]). To directly establish the stoichiometry of Na−SO_4 influx, it would be neces-

sary to determine not only the Na-dependence of SO_4 influx but also the SO_4-dependence of Na influx. The latter would be very difficult to determine, however, since there are other, larger avenues of Na entry. Since SO_4 transport is electrically neutral, a transport stoichiometry of 2 Na per SO_4 seems likely. The possibility that a second cation is or can be transported along with Na and SO_4 has not been explored, however. The $Na-SO_4$ cotransport system appears to be distinct from the previously described Na−Cl cotransport [25]. This is attested to both by the rather modest inhibition of SO_4 influx produced by a large excess of Cl and by the failure of secretory stimuli such as theophylline, which completely inhibits Na−Cl cotransport [25], to inhibit Na-dependent SO_4 influx.

Our observations agree with the recently reported observations of Lücke et al. [23] on SO_4 uptake by ileal brush border vesicles. They found that Na stimulated uptake and produced a typical "overshoot" phenomenon but that K, Li, Rb, and Cs did not. They also concluded that SO_4 uptake is electrically neutral since it was not altered by varying the PD across the vesicular membrane. Finally, they found greater uptake into ileal than into jejunal or duodenal brush border vesicles. $Na-SO_4$ cotransport appears also to be present in mammalian kidney: micropuncture experiments have demonstrated a Na-dependent, ouabain-inhibitable mechanism for SO_4 absorption in the proximal tubule [34]; in experiments with renal cortical brush border vesicles, $Na-SO_4$ cotransport has been demonstrated and found to have the same characteristics as the ileal brush border process [22].

Cl-Dependent SO_4 Efflux Across the Basolateral Membrane

Net transepithelial SO_4 transport can be almost completely inhibited by replacing all Cl with gluconate or by adding SITS or DIDS on the serosal side. The inhibitory effect of Cl removal is clearly not exerted on the brush border entry step since SO_4 influx was found to increase in the absence of Cl. Furthermore, serosally added SITS did not diminish SO_4 influx and it tripled intracellular SO_4 concentration. Its effect, therefore, must have been exerted on the serosal exit step. The presence in the basolateral membrane of an electrically neutral exchange mechanism with high affinities for SO_4 and Cl is not really surprising in view of the demonstrated presence of such a mechanism in erythrocytes [21] and in Ehrlich ascites tumor cells [35] and in view of the electrophysiological evidence for low ionic conductivity in the basolateral membranes of comparable epithelia (flounder intestine [33], rabbit [7] and *Necturus* [28] gallbladder).

The observed suceptibility of the serosal border SO_4 transport process to inhibition by SITS and DIDS is also consistent with the recent observations of Grinstein et al. [16] that SO_4 efflux from basolateral membrane vesicles prepared from either renal cortex or small intestine is inhibited by 50 μM DIDS. In contrast,they found little or no inhibition by DIDS of SO_4 efflux from brush border vesicles. This is consistent with our own observation that SITS (50 μM), when added on the mucosal side, does not inhibit SO_4 transport.

The effects of both Cl removal and SITS on the *s*-to-*m* unidirectional SO_4 flux indicate that the majority of this flux is transcellular and, more specifically, traverses the serosal border by anion exchange. The striking increase in *s*-to-*m* SO_4 flux produced by removal of Cl suggests competitive interaction between Cl and SO_4. Most of this increase in *s*-to-*m* flux is probably due to SO_4 self-exchange. The anionic specificities of the serosal border process are further defined in the following paper [20]. The inhibitory effect of SITS on the *s*-to-*m* flux indicates that 75% of this flux is transcellular. If we assume that the SITS-independent *s*-to-*m* flux is paracellular, then the apparent permeability of the paracellular pathway to SO_4 is $0.018/2.35 = 7.7 \times 10^{-3}$ cm hr^{-1}. This is about 10% of the apparent paracellular Na permeability as estimated from the *s*-to-*m* Na flux [26].

Since SO_4/Cl exchange appears to be the major, if not the only, avenue for net SO_4 transport across the basolateral membrane, it is pertinent to ask whether the medium-to-cell Cl concentration difference provides part of the driving force for net SO_4 absorption. Although Cl-selective microelectrode measurements on rabbit ileum have not been reported, measurements with ^{36}Cl of the exchangeable pool of Cl suggest that intracellular Cl concentration is less than half of the extracellular concentration [13]. Our measurement of intracellular SO_4 concentration, based on $^{35}SO_4$ equilibration, was 1.1 mM at a medium concentration of 2.4 mM. Even if very little SO_4 is present in crypt cells, which constitute about 40% of the epithelium [18], SO_4 activity in the absorbing cells would still be less than in the medium. The Cl concentration difference may therefore provide the driving force for uphill SO_4 movement by an electrically neutral SO_4/Cl exchange.

Effects of Secretory Stimuli on SO_4 Transport

A major purpose of the present study was to learn more about the intestinal secretory process by determining the effects of secretory stimuli on the transport of an inorganic anion other than a halide. Cyclic AMP is thought to modify ileal water and electrolyte

transport at two separate sites [12]: (1) it inhibits Na−Cl cotransport across the brush border, and (2) it stimulates active Cl secretion, probably in crypt cells. The latter process appears to involve a Na−Cl cotransport across the basolateral membrane and a Cl "gate" in the luminal membrane that is regulated by cAMP and Ca. We know very little about the precise biochemical basis for these regulatory effects (i.e., which membrane of cell proteins are phosphorylated, what the functions of the phosphorylated proteins are, etc.). It was of interest, therefore, to determine whether cAMP or other secretory stimuli would inhibit the absorption or even stimulate the secretion of SO_4. The results indicate that, although modest inhibition of SO_4 absorption was observed, the cellular effects of these secretory stimuli seem to be specific for Cl. Thus Na−SO_4 cotransport across the brush border was not inhibited. Furthermore, no net SO_4 secretion developed and the *s*-to-*m* flux did not increase.

The modest simulation of Na-dependent SO_4 influx produced by theophylline and probably also heat-stable enterotoxin (ST) may represent a specific effect of cGMP. Theophylline increases cGMP as well as cAMP concentration and ST increases only cGMP [10]. In contrast to the effects of theophylline and ST, 8-Br-cAMP was not stimulatory. The number of relevant measurements is small, however, and more experiments need to be done to verify and extend this observation.

The partial inhibition of net SO_4 absorption produced by several secretory stimuli remains to be explained. This inhibition could have an extracellular origin. Since these secretagogues inhibit salt and water absorption as well as stimulating their secretion, they decrease both volume and flow rate in the lateral intercellular spaces of the villus region. As one consequence, SO_4 concentration would tend to build up there, increasing the work needed to effect net SO_4 transport from cell to lateral space. Theophylline has previously been shown to inhibit ileal amino acid absorption by about one-third [15], which is comparable to the observed inhibition of SO_4 absorption. The explanation may be the same in both instances.

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