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Mechanisms of oxalate absorption and secretion across the rabbit distal colon

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Abstract. To further evaluate the mechanisms of oxalate (Ox^{2-}) transport in the intestine the following studies were performed using isolated, short-circuited segments of the rabbit distal colon (DC). In control buffer, the DC absorbed Ox^{2-} (net Ox^{2-} flux, $J^{Ox}_{Net} = 5.4 \pm 0.7$ pmol · $cm^{-2} \cdot h^{-1}$). Replacement of Na^+ with *N*-methyl-D-glucamine ($NMDG^+$) abolished Ox^{2-} absorption by decreasing mucosal to serosal Ox^{2-} flux (J^{Ox}_{ms}), without affecting Cl^- transport, while gluconate substitution for Cl^- did not affect J^{Ox}_{Net} or net Na^+ flux (J^{Na}_{Net}). Addition of Na^+ to the serosal side of tissues bathed by $NMDG^+$ buffer increased J^{Ox}_{ms} 40% without altering mucosal to serosal Cl^- flux (J^{Cl}_{ms}). Serosal amiloride or dimethyl amiloride (10^{-3} M) abolished J^{Ox}_{Net} by decreasing J^{Ox}_{ms}, it increased serosal to muscosal Cl^- flux (J^{Cl}_{sm}) and it gradually inhibited short-circuit current (I_{sc}). Mucosal amiloride (10^{-4} M) abolished I_{sc} but had no effect on Ox^{2-} or Cl^- fluxes. Serosal 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 10^{-6} M) reduced J^{Ox}_{ms} by 20% and J^{Ox}_{Net} by 43% without affecting J^{Cl}_{ms} or J^{Cl}_{Net}. Dibutyryl cyclic adenosine monophosphate (dB-cAMP, 5×10^{-4} M, both sides) stimulated Ox^{2-} secretion ($J^{Ox}_{Net} = -12.6 \pm 3.3$ pmol · $cm^{-2} \cdot h^{-1}$). The dB-cAMP-induced secretion of Ox^{2-} and Cl^- were fully abolished by serosal furosemide (10^{-4} M) and partially inhibited (35%) by 5×10^{-4} M mucosal NPPB [5-nitro-2-(3-phenylpropylamino)-benzoic acid], a putative Cl^- channel blocker. It is proposed that: (1) basal absorption of Ox^{2-}, but not Cl^-, is dependent upon a previously undescribed basolateral Na^+-H^+ exchanger that may be coupled to a DIDS-sensitive, basolateral anion exchange system that mediates Ox^{2-} flux; (2) the DC secretes Ox^{2-} in response to dB-cAMP by a mechanism that is indistinguishable from the pathway for Cl^- secretion.

Key words: Sodium – Chloride – cAMP – DIDS – Furosemide – NPPB – Amiloride – Na^+-H^+ exchange – Anion exchange – Basolateral membrane

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Introduction

It has been estimated that the fraction of dietary oxalate absorbed by calcium oxalate stone-formers is more than three times the normal level and that some of these individuals must by hyperabsorbing oxalate [9]. The principal site for the hyperabsorption of dietary oxalate in individuals with enteric hyperoxaluria is the colon and a secondary clinical consequence of this is oxalate nephrolithiasis [5, 8]. In a recent study comparing oxalate handling by various segments of the rabbit intestine [14], it was observed that the distal colon is unique in supporting a net absorptive flux of oxalate. In contrast, the other intestinal segments were shown to secrete oxalate, providing a potential extrarenal excretory route for oxalate. It is now conceivable that dietary or absorptive hyperoxaluria may be a consequence of a decrease in the secretory component of intestinal oxalate transport, as well as an enhancement of the absorptive component. Consequently, the mechanisms involved in the handling of oxalate by the intestine, particularly colonic oxalate absorption, are important considerations not only in the management of hyperoxaluria and oxalate stone disease but also in oxalosis that can occur in chronic renal failure [2, 5, 8, 9].

Results from early investigations into the mechanism of intestinal oxalate absorption suggested that transepithelial oxalate fluxes were passive [2, 5, 8]. Later, in vitro preparations of rat [10] and rabbit [15] distal colon were observed to support net oxalate absorption and, in the rabbit, the absorptive flux was sensitive to metabolic and transport inhibitors. In the rabbit, the net movements and sensitivities of oxalate appeared to parallel those of chloride, hence it was postulated that oxalate absorption was a mediated process and oxalate competed with chloride for sites on the Cl^--HCO_3^- exchanger in the apical membrane of colonocytes [15]. The proposal that anion exchangers can mediate transmembrane oxalate fluxes is supported, in part, by recent descriptions of apical membrane Ox^{2-}-OH^- and Ox^{2-}-Cl^- exchangers [20] and basolateral SO_4^{2-}-HCO_3^- (Ox^{2-}) exchangers

[19] in membrane vesicles from the rabbit ileum and Cl^--OH^-/Cl^--HCO_3^- exchangers in rat colon apical membrane vesicles that are cis inhibited by oxalate [23].

While these details of oxalate exchange mechanisms are being revealed, it is still vague as to how these apical and basolateral transporters are coordinated in any intestinal segment to produce net transepithelial oxalate movements. Furthermore, the degree (if any) to which net oxalate transport can be regulated has not been addressed. In the present report on the isolated rabbit distal colon we present the results of studies that consider some of these questions. We found that the mucosal to serosal component of oxalate absorption exhibited a marked sodium sensitivity and was partially inhibitable by serosal amiloride, ouabain, and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS). This result suggests that oxalate efflux across the basolateral membrane is mediated by an exchange mechanism that is possibly coupled to a previously undescribed Na^+-H^+ exchanger in this membrane. Additionally, net oxalate absorption was shown to be readily converted to net secretion by a cyclic adenosine monophosphate (cAMP) analogue. This induced secretory pathway paralleled that of chloride in being blocked by serosal furosemide and in exhibiting sensitivity to mucosal application of the putative chloride channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) [4, 6].

Materials and methods

The following studies were performed using 2- to 3-kg, male, New Zealand White rabbits with the approval of the Animal Research Committee of the University of California at Irvine. Rabbits were maintained on a 12-h light/12-h dark photoperiod and were used 4–5 h after the onset of light. After an intravenous overdose of sodium pentobarbital, a 10-cm segment of the distal colon, starting at the base of the urinary bladder, was removed, opened along the mesenteric border, rinsed with 0.9% NaCl, and placed in chilled standard buffer (described below). Epithelial tissues were stripped of serosal layers with glass microscope slides and spread over mounting frames as described previously [10]. The framed tissues were positioned in Ussing-type chambers having an aperture of 1.13 cm^2 and bathed by 10 ml buffer solution on each side. Each reservoir was maintained at 37°C and the buffer solutions were circulated with a gas lift system.

With the exception of the ion replacement studies, all experiments were performed with a standard buffered saline containing the following solutes (in mM): 139.4 Na^+; 123.2 Cl^-; 5.4 K^+; 1.2 Ca^{2+}; 1.2 Mg^{2+}; 21 HCO_3^-; 0.6 $H_2PO_4^-$; 2.4 HPO_4^{2-}; and 10 glucose. The total concentration of oxalate in these solutions was 1.5 μM. Sodium-free solutions were prepared by replacing the Na^+ with *N*-methyl-D-glucamine ($NMDG^+$) and chloride-free buffers were made by substituting gluconate for chloride. These solutions were gassed with a 95% O_2/5% CO_2 mixture. In experimental conditions, all buffers had pH of 7.4 ± 0.05. Reagent grade salts for these solutions were purchased from Sigma Chemical (St. Louis, Mo., USA) as were amiloride, DIDS, furosemide, and dibutyryl cAMP (dB-cAMP). Dr. R. Greger, University of Freiburg, generously provided the NPPB.

Unidirectional fluxes were measured using isotopic techniques on tissues which were continuously short-circuited, except when measuring open-circuit potential (V_t), with voltage clamps (VCC600, Physiological Instruments) that corrected for series solution resistance. Isotopes (^{14}C-oxalate and either ^{36}Cl or ^{22}Na) were added to one reservoir 30 min after mounting the tissues, and sampling from the opposing reservoir commenced 30 min thereafter. At the appropriate time, 1 ml of the "cold" reservoir solution was removed and replaced with fresh, unlabelled buffer and the electrical parameters were recorded. Aliquots of the "hot" reservoir solution were taken at the beginning and end of experiment and averaged to obtain specific activities. Samples were dissolved in EcoScint (National Diagnostics, Manville, N. J., USA) and isotopic activity was measured with quench correction with a Beckman 9000 liquid scintillation spectrometer. Unidirectional solute fluxes were computed (J^{solute} = cpm/time · specific activity · area) and expressed as pmol (for oxalate) or μEq · cm^{-2} · h^{-1} (for Na^+ and Cl^-). Net fluxes were calculated as the difference in the unidirectional fluxes between adjacent tissues having similar conductances. Short-circuit current (I_{sc}, μA) and V_t were recorded at the start and end of each sampling interval and averaged for that interval. I_{sc} is expressed in μEq · cm^{-2} · h^{-1} and tissue conductance (G_t, from Ohm's Law) in mS · cm^{-2}.

In this study up to three experimental periods (periods I–III) were employed, where each period represents the mean of three consecutive sampling intervals as described above. Each period was preceded by a 20-min "equilibration" interval, during which experimental drugs, if any, were added.

Results are presented as the mean of n tissues or tissue pairs ± 1 SEM. The significance of differences between means was evaluated using Student's t-test (two-tailed) for paired or unpaired comparisons, as indicated. Comparison of more than two means was made using a one-way analysis of variance in conjunction with Duncan's multiple range test. For all comparisons, differences were judged significant if $P \leq 0.05$.

Results

Oxalate absorption

To establish the ionic dependencies of net oxalate transport across the rabbit distal colon, a series of ion substitution experiments were performed. As shown in Table 1, in the standard (control) buffer the distal colon exhibited a net absorption of oxalate, sodium, and chloride. I_{sc} was not significantly different from net sodium absorption, indicating that the bulk of the current is due to electrogenic sodium absorption as is characteristic of this tissue in these conditions [12]. Throughout the course of these studies, the unidirectional fluxes of chloride and oxalate exhibited a variability of up to twofold in control conditions. This variability was not correlated with variations in G_t, suggesting that this was part of the spontaneous variability of these transport systems and not due to variations in tissue preparation. In all control conditions a net absorption of oxalate was observed.

Replacement of sodium with $NMDG^+$ abolished J^{Ox}_{Net} by significantly depressing J^{Ox}_{ms} (Table 1). Chloride fluxes were unaffected by sodium removal, but G_t decreased reflecting the fact that I_{sc} was not different from zero in the absence of sodium. The sodium dependency of J^{Ox}_{Net} was also observed when the distal colon was treated with serosal ouabain (10^{-4} M). In eight tissue pairs, ouabain abolished net oxalate absorption (from 7.9 ± 1.6 to 0.4 ± 1.2 pmol · cm^{-2} · h^{-1}). When gluconate replaced chloride, both unidirectional oxalate fluxes increased, but only the change in J^{Ox}_{sm} was significant (Table 1). In contrast, sodium fluxes and the transepithelial electrical properties were not significantly altered by chloride removal.

Table 1. Sodium and chloride dependence of oxalate transport across the rabbit distal colon

Conditions	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride and sodium fluxes (μEq · cm^{-2} · h^{-1})									I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J^{Ox}_{sm}	J^{Ox}_{ms}	J^{Ox}_{Net}	J^{Cl}_{sm}	J^{Cl}_{ms}	J^{Cl}_{Net}	J^{Na}_{sm}	J^{Na}_{ms}	J^{Na}_{Net}		
Control (11,5,6**)	6.2 ± 1.2	11.6 ± 1.8	5.4 ± 0.7	2.66 ± 0.53	4.51 ± 0.61	1.85 ± 0.21	0.38 ± 0.09	1.54 ± 0.11	1.16 ± 0.21	1.23 ± 0.21	2.48 ± 0.19
Na^+-free (5)	6.1 ± 0.7	6.2* ± 1.1	0.1* ± 0.5	2.35 ± 0.39	3.81 ± 0.66	1.45 ± 0.34				0.11* ± 0.02	0.86* ± 0.05
Cl^--free (8)	11.8* ± 1.5	15.3 ± 2.5	3.5 ± 1.2				0.52 ± 0.09	2.08 ± 0.46	1.56 ± 0.37	1.94 ± 0.35	1.90 ± 0.35

J^{Ox}_{sm}, Oxalate flux, serosal to mucosal; J^{Ox}_{ms}, oxalate flux, mucosal to serosal; J^{Ox}_{Net}, net oxalate flux; J^{Cl}_{ms}, Cl^- flux, mucosal to serosal; J^{Cl}_{Net}, net Cl^- flux; J^{Na}_{sm}, Na^+ flux, serosal to mucosal; J^{Na}_{ms}, Na^+ flux, mucosal to serosal; J^{Na}_{Net}, net Na^+ flux; I_{sc}, short-circuit current; G_t tissue conductance
(n), number of tissue pairs
In control condition, ** indicates (n) for Cl^- and Na^+ fluxes
* Significant difference from control ($P \leq 0.05$) based upon an unpaired t-test

Table 2. The effect of serosal Na^+ addition on oxalate and Cl^- absorptive fluxes in rabbit distal colon initially bathed by Na^+-free buffers

Condition	J^{Ox}_{ms}	J^{Cl}_{ms}	I_{sc}	G_t
	(pmol · cm^{-2} · h^{-1})	(μEq · cm^{-2} · h^{-1})		(mS · cm^{-2})
$NMDG^+$ (M + S)	14.2 ± 1.3	3.23 ± 0.33	0.17 ± 0.5	1.94 ± 0.28
+ Na^+ (S)	20.0* ± 2.2	3.24 ± 0.33	0.34* ± 1.4	3.14* ± 0.33

$NMDG^+$, *N*-Methyl-D-glucamine. M and S refer to the mucosal and serosal buffer, respectively. Results from 9 tissue pairs (n) from 5 animals
* Significant difference from control ($P \leq 0.05$) based upon a paired t-test

In the rabbit distal colon sodium influx from the luminal solution into the enterocyte is mediated exclusively by apical membrane sodium channels [1, 12]. Hence, to localize the sodium dependency of J^{Ox}_{Net} noted above we focused on the possibility that J^{Ox}_{ms} was dependent upon sodium in the serosal bath. To test this we measured J^{Ox}_{ms} and J^{Cl}_{ms} in tissues initially bathed by sodium-free buffer as in Table 1 and then replaced the serosal $NMDG^+$ buffer with standard Na^+-containing saline and remeasured anion fluxes in period II. As shown in Table 2, addition of Na^+ to the serosal side produced a significant 40% increase in J^{Ox}_{ms} without affecting J^{Cl}_{ms}. The attendant changes in electrical properties of the tissue in period II are likely due to uncompensated changes in solution resistance, sodium $NMDG^+$ diffusion potentials, and sodium gaining access to apical membrane sodium conductance pathways. The important point is that sodium addition to the serosal side increased J^{Ox}_{ms} without affecting J^{Cl}_{ms}. As chloride transport was unaltered, the increase in oxalate flux can not be attributed to an overall increase in tissue permeability associated with the physical maneuver of flushing the serosal compartment.

The sidedness and nature of the sodium dependence of J^{Ox}_{ms} were further examined by measuring oxalate and chloride fluxes in standard saline in the absence and presence of serosal amiloride and dimethyl amiloride. At the dosages employed here (10^{-3} M) amiloride and its analogues are generally, albeit arguably[1], considered to inhibit Na^+-H^+ exchange [1, 26]. As shown in Table 3, serosal amiloride and dimethyl amiloride blocked net oxalate absorption by significantly decreasing J^{Ox}_{ms}, while J^{Ox}_{sm} was unaffected. Both of the amilorides produced small reductions in the unidirectional chloride fluxes, but only the change in J^{Cl}_{sm} was significant. I_{sc} and G_t fell throughout the first two flux intervals of Period II, suggesting that the amilorides had gained access to the mucosal reservoir and were blocking apical membrane sodium channels. In another series (Table 4), addition of a low dose of amiloride (10^{-4} M) to the mucosal solution bathing control tissues had no effect on the unidirectional or net fluxes of oxalate or chloride, but did inhibit I_{sc} (electrogenic sodium absorption). Thus, J^{Ox}_{ms} is not dependent on sodium entry across the apical membrane and the effect of serosal amiloride and dimethyl amiloride appears localized to a basolateral membrane exchange process.

Interpretation of the basolateral sodium dependence of oxalate absorption in terms of a Na^+-H^+ exchange system implies that oxalate flux across the basolateral membrane may be mediated by an anion exchange system that is dependent upon the activity of the amiloride-sensitive pathway. To evaluate the possibility that basolateral oxalate efflux is associated with an anion exchange system, we determined the effects of serosal application of the disulfonic stilbene, DIDS, on oxalate and chloride transport across the rabbit distal colon. The results of this series of experiments are presented in Table 5. At a serosal concentration of 10^{-6} M, DIDS decreased J^{Ox}_{ms} 20% which resulted in a 43% fall in J^{Ox}_{Net},

[1] A common interpretation of the pharmacological action of millimolar concentrations of amiloride and its analogues is that they inhibit Na^+-H^+ antiporters [1, 18, 26]. In light of the fact that other Na^+ transport pathways can be affected by amiloride [29] and because we were unable to evaluate other criteria for establishing Na^+-H^+ antiport using the methods employed here, conclusions regarding the nature of the observed amiloride sensitivity of oxalate absorption must be considered to be tentative.

Table 3. The effect of serosal amiloride and dimethyl amiloride on oxalate and NaCl transport across the rabbit distal colon

Conditions	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride and sodium fluxes (μEq · cm^{-2} · h^{-1})									I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J^{Ox}_{sm}	J^{Ox}_{ms}	J^{Ox}_{Net}	J^{Cl}_{sm}	J^{Cl}_{ms}	J^{Cl}_{Net}	J^{Na}_{sm}	J^{Na}_{ms}	J^{Na}_{Net}		
Control (14,8,6)**	10.0 ± 1.6	17.9 ± 2.1	7.9 ± 1.1	2.53 ± 0.20	3.46 ± 0.40	0.93 ± 0.37	0.41 ± 0.08	1.63 ± 0.28	1.21 ± 0.20	0.67 ± 0.07	2.36 ± 0.22
+ Amiloride	10.6 ± 0.3	11.6* ± 0.3	1.0* ± 1.1	2.14* ± 0.23	3.20 ± 0.28	1.05 ± 0.36	0.42 ± 0.05	0.69* ± 0.16	0.27* ± 0.14	− 0.08* ± 0.03	1.80* ± 0.17
Control (9)	11.1 ± 1.0	16.3 ± 0.9	5.2 ± 1.0	2.25 ± 0.24	2.93 ± 0.14	0.68 ± 0.22	–	–	–	0.46 ± 0.18	2.26 ± 0.19
+ Dimethyl amiloride	10.7 ± 0.9	12.3* ± 0.6	1.6* ± 0.5	1.84* ± 0.18	2.59 ± 0.17	0.75 ± 0.17	–	–	–	0.14* ± 0.08	1.96* ± 0.12

Amiloride or dimethyl amiloride (10^{-3} M) was added to the serosal side only
* Significant difference from control ($P \leq 0.05$) based upon a paired *t*-test
** Results for oxalate and electrical parameters are from 14 tissue pairs (from 7 animals) where 8 included $^{36}Cl^-$ and 6 included $^{22}Na^+$, and 9 tissue pairs from 3 animals in the dimethyl amiloride series

Table 4. The effect of mucosal amiloride on oxalate and Cl^- transport across the rabbit distal colon

Condition	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride flux (μEq · cm^{-2} · h^{-1})						I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J^{Ox}_{sm}	J^{Ox}_{ms}	J^{Ox}_{Net}	J^{Cl}_{sm}	J^{Cl}_{ms}	J^{Cl}_{Net}		
Control	9.0 ± 1.3	14.2 ± 0.3	5.2 ± 0.5	1.57 ± 0.14	2.63 ± 0.28	1.06 ± 0.18	1.01 ± 0.13	1.82 ± 0.14
+ Amiloride	8.8 ± 1.3	14.6 ± 1.9	5.8 ± 1.4	1.63 ± 0.15	2.86 ± 0.37	1.23 ± 0.24	0.14* ± 0.06	1.55 ± 0.21

Results from 8 tissue pairs (*n*) from 5 animals. Amiloride (10^{-4} M) was added to the mucosal side only
* Significant difference from control ($P \leq 0.05$) based upon a paired *t*-test

Table 5. The effect of serosal DIDS (10^{-6} M) on oxalate and Cl^- transport across the rabbit distal colon

Condition	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride flux (μEq · cm^{-2} · h^{-1})						I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J^{Ox}_{sm}	J^{Ox}_{ms}	J^{Ox}_{Net}	J^{Cl}_{sm}	J^{Cl}_{ms}	J^{Cl}_{Net}		
Control	8.0 ± 0.9	14.3 ± 0.9	6.3 ± 1.0	1.50 ± 0.10	2.39 ± 0.26	0.90 ± 0.21	0.65 ± 0.09	1.74 ± 0.12
+ DIDS (S)	7.8 ± 0.9	11.4* ± 1.1	3.6* ± 1.4	1.22* ± 0.10	2.02 ± 0.26	0.98 ± 0.18	0.51* ± 0.07	1.61* ± 0.11

Results from 7 tissue pairs (*n*) from 4 animals
* Significant difference from control ($P \leq 0.05$) based upon a paired *t*-test

as the serosal to mucosal flux was not significantly affected. Decreases in J^{Cl}_{sm} and tissue electrical properties were also noted, but J^{Cl}_{ms} was not significantly affected by 10^{-6} M serosal DIDS. Addition of 10^{-6} M DIDS to the mucosal side of the tissues had no effect on anion fluxes or electrical properties of the tissues (data not shown), suggesting that serosal DIDS was affecting only a basolateral transport process. At a higher concentration of serosal DIDS (10^{-5} M) we did observe a more complete inhibition of J^{Ox}_{ms} (from 15.2 ± 2.2 to 7.7 ± 0.3 pmol · cm^{-2} · $^{-1}$), but J^{Cl}_{ms} was also significantly reduced (2.93 ± 0.27 to 2.03 ± 0.18 μEq · cm^{-2} · h^{-1}). Since the mucosal to serosal fluxes of both anions are sensitive to higher mucosal concentrations of disulfonic stilbenes ([15] and unpublished observations), the side of the epithelium affected by the higher dosages of DIDS is uncertain. In any event, the partial reduction in the absorptive flux of oxalate, but not chloride, observed in this series at 10^{-6} M DIDS further supports the hypothesis presented above that these anions may cross the basolateral membrane by distinct mechanisms. Furthermore, the sensitivity of J^{Ox}_{ms} to low concentrations of DIDS would indicate that oxalate efflux across this barrier may be partially mediated by an anion exchange system.

Oxalate secretion

The distal colon secretes chloride and potassium in response to a variety of secretagogues [10, 13, 16, 21]. Given the correlation between chloride and oxalate fluxes noted here and elsewhere [14, 15], the effects of the permeable cAMP analogue dB-cAMP and inhibitors of chloride secretion were examined with respect to oxalate transport and are presented below.

Since the following experimental design included three periods, any time-dependent changes in dB-cAMP-

Table 6. The effects of dibutyryl cyclic adenosine monophosphate (dB-cAMP) on oxalate and Cl^- transport across the rabbit distal colon

Condition	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride flux (μEq · cm^{-2} · h^{-1})						I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J_{sm}^{Ox}	J_{ms}^{Ox}	J_{Net}^{Ox}	J_{sm}^{Cl}	J_{ms}^{Cl}	J_{Net}^{Cl}		
Control	13.1 ± 3.7	18.8 ± 1.3	5.8 ± 2.9	2.16 ± 0.34	3.42 ± 0.23	1.25 ± 0.33	0.71 ± 0.14	2.30 ± 0.40
+ dB-cAMP	36.2* ± 10.6	16.3 ± 2.2	− 19.9* ± 8.8	6.83* ± 1.40	3.09 ± 0.16	− 3.74* ± 1.28	4.13* ± 0.64	5.71* ± 0.64
+ dB-cAMP	38.0* ± 14.1	14.8 ± 1.1	− 23.2* ± 14.6	6.76* ± 1.81	2.58* ± 0.25	− 4.18* ± 2.04	2.95* ± 0.65	4.60** ± 0.87

Results from 5 tissue pairs (n) from 4 animals
* Significant difference from controls (period I), ** significant different from period II based upon ANOVA ($P \leq 0.05$)

Table 7. The effects of furosemide on dB-cAMP-induced secretion of oxalate and Cl^- across the rabbit distal colon

Condition	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride flux (μEq · cm^{-2} · h^{-1})						I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J_{sm}^{Ox}	J_{ms}^{Ox}	J_{Net}^{Ox}	J_{sm}^{Cl}	J_{ms}^{Cl}	J_{Net}^{Cl}		
Control	12.5 ± 3.1	16.1 ± 2.6	3.6 ± 1.1	1.84 ± 0.36	2.73 ± 0.32	0.89 ± 0.16	0.72 ± 0.07	1.82 ± 0.38
+ dB-cAMP	40.5* ± 10.0	15.1 ± 1.1	− 25.4* ± 8.9	7.32* ± 1.25	2.38 ± 0.16	− 4.93* ± 1.19	4.30* ± 0.34	5.11* ± 0.35
+ Furosemide	16.3** ± 3.8	12.7 ± 1.6	− 3.6** ± 2.6	2.86** ± 0.47	2.21 ± 0.10	− 0.65** ± 0.40	1.51*,** ± 0.18	3.00*,** ± 0.50

Results from 5 tissue pairs (n) from 3 animals. Furosemide was added to the serosal side at 10^{-4} M
* Significant difference from controls (period I), ** significant difference from period II based upon ANOVA ($P \leq 0.05$)

Table 8. The effects of furosemide pretreatment on the ability of dB-cAMP to stimulate oxalate and Cl^- transport across the rabbit distal colon

Condition	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride and sodium fluxes (μEq · cm^{-2} · h^{-1})						I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J_{sm}^{Ox}	J_{ms}^{Ox}	J_{Net}^{Ox}	J_{sm}^{Cl}	J_{ms}^{Cl}	J_{Net}^{Cl}		
Control	11.0 ± 1.6	16.8 ± 1.5	5.8 ± 1.5	2.69 ± 0.77	4.29 ± 1.11	1.60 ± 0.42	1.06 ± 0.48	2.13 ± 0.22
+ Furosemide	8.3 ± 0.7	17.8 ± 1.7	9.5 ± 2.1	2.05* ± 0.63	3.94 ± 1.06	1.89 ± 0.51	0.89 ± 0.32	1.76 ± 0.19
+ dB-cAMP	10.2 ± 1.0	13.6*,** ± 2.0	3.5** ± 1.2	2.26* ± 0.58	3.08* ± 0.64	0.81** ± 0.18	1.21 ± 0.29	2.78** ± 0.18

Results from 6 tissue pairs (n) from 5 animals. Furosemide was added to the serosal side at 10^{-4} M
* Significant difference from control (period I), ** significant difference from period II based upon ANOVA ($P \leq 0.05$)

stimulated transport were first assessed. As shown in Table 6, dB-cAMP (0.5 mM, both sides) added between periods I and II reversed oxalate and chloride absorption to net secretion. These changes in net transport for both ions were due to significant increases in the serosal to mucosal fluxes. I_{sc} and G_t increased significantly due to electrogenic chloride secretion [16]. Chloride and oxalate secretion persisted for over 2 h, whereas I_{sc} and G_t fell in period III, which most likely reflects the establishment of net potassium secretion [10, 13, 21]. These results indicate that dB-cAMP stimulates a marked secretion of oxalate as well as chloride and both fluxes are sufficiently constant through time to evaluate the effects of inhibitors applied between periods II and III.

The dB-cAMP-induced secretion of both oxalate and chloride were blocked by serosal furosemide (10^{-4} M), a loop diuretic that blocks Na^+-K^+-$2Cl^-$ cotransport [22, 31]. As shown in Table 7, furosemide reduced dB-cAMP-induced increases in J_{sm}^{Ox} by 40% and J_{sm}^{Cl} by 60% without affecting the mucosal to serosal components. Both I_{sc} and G_t were also significantly reduced by furosemide by virtue of the inhibition of electrogenic chloride secretion. In another series of experiments (Table 8), furosemide (10^{-4} M, serosal) was added in period II and dB-cAMP in period III to further assess furosemide sensitivity. Furosemide had no effect on the unidirectional or net fluxes of the control (absorbing) tissues. Addition of dB-cAMP affected only the mucosal to serosal components, depressing J_{ms}^{Ox} 28% and J_{ms}^{Cl} 26%, thereby depressing the respective net absorptive fluxes.

These experiments indicate that oxalate secretion can, indeed, be induced in the rabbit distal colon. The

Table 9. The effects of NPPB on dB-cAMP-stimulated oxalate and Cl^- transport across the rabbit distal colon

Condition	Oxalate flux (pmol · cm^{-2} · h^{-1}), chloride flux (μEq · cm^{-2} · h^{-1})						I_{sc} (μEq · cm^{-2} · h^{-1})	G_t (mS · cm^{-2})
	J^{Ox}_{sm}	J^{Ox}_{ms}	J^{Ox}_{Net}	J^{Cl}_{sm}	J^{Cl}_{ms}	J^{Cl}_{Net}		
Control	9.4 ± 0.9	16.8 ± 1.8	7.4 ± 2.2	1.95 ± 0.17	3.50 ± 0.37	1.55 ± 0.42	1.07 ± 0.21	1.83 ± 0.26
+ dB-cAMP	27.2* ± 2.0	14.5 ± 1.6	− 12.6* ± 3.0	5.81* ± 0.45	2.95* ± 0.27	− 2.86* ± 0.67	4.14* ± 0.37	4.62* ± 0.29
+ NPPB	17.4** ± 3.1	11.3 ± 2.0	− 6.1** ± 4.2	3.83** ± 0.68	2.08** ± 0.32	− 1.75** ± 0.98	0.65** ± 0.28	3.37** ± 0.11

Results from 6 tissue pairs (n) from 5 animals. NPPB was dissolved in dimethyl sulfoxide (DMSO) and added to the mucosal side at a final concentration of 5×10^{-5} M. The DMSO vehicle had no effect on electrical or transport characteristics of parallel control tissues
* Significant difference from controls (period I), ** significant difference from period II based upon ANOVA ($P \leq 0.05$)

furosemide sensitivity of this process suggests the possibility that oxalate utilizes a similar, if not the same, secretory pathway as that for chloride. To further test this hypothesis, the effect of mucosal NPPB, a putative chloride channel blocker in secretory epithelia [4, 6, 17], was evaluated by adding this inhibitor between periods II and III to dB-cAMP-stimulated tissues. As presented in Table 9, addition of NPPB (0.5×10^{-5} M) to the mucosal side significantly reduced J^{Ox}_{sm} by 38% and J^{Cl}_{sm} by 34% and these changes resulted in a significant depression in dB-cAMP-induced net secretion of oxalate and chloride. These findings are in accord with a blockade of an apical membrane chloride conductance pathway that also accommodates the oxalate anion. The fall in I_{sc} is also suggestive of a decrease in electrogenic chloride secretion, but the decrease is larger than the change in J^{Cl}_{Net} indicating that other electrogenic pathways may also be affected by this inhibitor. Combined, these results indicate that NPPB effects are likely more complex than simply blocking apical membrane chloride conductance in the rabbit distal colon. Nonetheless, the similarity of NPPB actions on chloride and oxalate secretory fluxes does suggest a common mechanism in the efflux of these anions across the apical membrane of secretory colonocytes.

Discussion

The findings presented in this report provide new information regarding the mechanism of transepithelial oxalate absorption by the distal colon and also establish that secretagogues can promote net secretory fluxes of oxalate across this tissue.

Mechanism of oxalate absorption

Net oxalate absorption has been previously described in the rat [11] and rabbit [15] distal colon. In the latter study, mucosal 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) abolished net oxalate and chloride absorption by reducing the respective mucosal to serosal fluxes which suggested that oxalate and chloride share a common, SITS-sensitive pathway for transport across the apical membrane. Since a Cl^--HCO_3^- exchanger mediates electroneutral chloride uptake by the rabbit distal colon [12], it was proposed that the first step in oxalate absorption involved the same Cl^--HCO_3^- exchange system [15]. The possibility that the oxalate anion can be transported by an anion exchanger has been supported by recent studies employing rabbit ileal brush-border membrane vesicles in which Ox^{2-}-Cl^- and Ox^{2-}-OH^- exchangers have been documented [20].

While the evidence presented above indicates that cellular uptake of oxalate from the lumen is a mediated process and parallels that of chloride, the nature of the basolateral exit step in the distal colon has not been previously addressed. This situation reflects the lack of information concerning basolateral anion transport pathways in colonic epithelia in general. The results of the present investigation do, however, suggest some new possibilities regarding basolateral oxalate transport in the rabbit colon. Four different experiments described here indicated that oxalate efflux from the enterocyte to the serosal saline is markedly sodium sensitive and distinct from the chloride exit mechanism. First, $NMDG^+$ replacement of Na^+ abolished oxalate absorption by decreasing J^{Ox}_{ms} to 53% of the control value without altering J^{Ox}_{sm}. Notably, chloride transport was not affected by sodium substitution, confirming the results of earlier studies [12]. Second, addition of Na^+ to the serosal reservoirs of tissues initially bathed by $NMDG^+$ buffers, produced a significant increase in J^{Ox}_{ms} without affecting J^{Cl}_{ms}. Third, serosal ouabain abolished oxalate absorption. Although chloride fluxes were not measured in this experiment, results from other studies indicate that chloride transport is not altered by serosal ouabain [12]. Fourth, serosal amiloride (10^{-3} M) abolished net oxalate absorption by reducing J^{Ox}_{ms} to 65% of controls without altering J^{Cl}_{ms}. At this dose amiloride blocks Na^+-H^+ exchange[1] [1, 26], which indicates that oxalate absorption, but not chloride absorption, is dependent upon the activity of a Na^+-H^+ counter-transport system in the rabbit colon. This finding additionally accounts for the sodium sensitivity of oxalate absorption described in the points noted above and is consistent with the independence of

chloride and sodium observed here and elsewhere [12, 14, 28].

While a Na^+-H^+ antiporter has been identified in a variety of epithelia [29] and characterized in basolateral membrane vesicles from the rat distal colon [7, 24], the present study is, to our knowledge, the first to provide pharmacological evidence for such an exchanger in the rabbit distal colon. At this point, however, we can only surmize the connection between oxalate absorption (J_{ms}^{Ox}) and a basolateral Na^+-H^+ exchange process. The simplest possibility is that the transcellular component of J_{ms}^{Ox} is mediated by an anion exchange process that is also involved in the acid-base regulation of these cells. Since J_{ms}^{Cl} was unaffected by serosal amiloride (or sodium replacement), it seems unlikely that the apical uptake mechanism [$Cl^-(Ox^{2-})$-HCO_3^-] is the sodium (H^+) sensitive step. However, a basolateral anion exchanger, such as the $(Ox^{2-})HCO_3^-$-SO_4^{2-} exchanger observed in rabbit ileal basolateral membrane vesicles [19], could account for the present findings. As considered below, the DIDS-sensitive component on the serosal aspect of the epithelium may contribute to this coupling scheme.

The absorptive component of oxalate flux, but not that of chloride, was partially reduced by low concentrations (10^{-6} M) of the anion exchange inhibitor DIDS when applied serosally. Mucosal addition of DIDS at this dose had no measurable effect on chloride or oxalate transport across the rabbit colon. One interpretation of these observations is that the DIDS-sensitive component of oxalate absorption may represent that portion of the anion exchange pathway that is coupled to the amiloride/sodium-sensitive component of J_{ms}^{Ox}. The fact that chloride flux was unaltered by serosal DIDS (10^{-6} M) and that chloride absorptive fluxes were also unaffected by maneuvers that affected sodium transport are consistent with this interpretation. Higher doses of serosal DIDS (10^{-5} M) produced a more pronounced inhibition of J_{ms}^{Ox}, yet this dose also produced a significant reduction in J_{ms}^{Cl}. At the present time we are unable to discern whether 10^{-5} M serosal DIDS also affects the stilbene-sensitive apical membrane anion exchanger [15] or some other chloride transport pathway [3, 28]. We can only conclude that at least part of the basolateral efflux of oxalate is mediated by a DIDS-sensitive mechanism that is distinct from that for chloride. Part or all of this DIDS-sensitive component may represent the proposed base-anion pathway which is coupled to the amiloride sensitive, sodium-dependent component of oxalate absorption. Clearly, additional studies are required to confirm these proposals.

Mechanism of oxalate secretion

Another principal finding presented here was that the distal colon exhibits a substantial capacity to secrete oxalate when presented with secretory stimuli. This aspect of the present study was prompted by a recent in vitro survey of oxalate handling by different parts of the rabbit intestine which showed net oxalate secretion by jejunum, ileum, and proximal colon under basal conditions [14] and by the fact that certain bile salts and long chain fatty acids stimulate oxalate secretion (unpublished observations).

To the extent examined here, net oxalate secretion by the rabbit distal colon was mediated by pathways that were fundamentally similar to those involved in the electrogenic secretion of chloride by this tissue. The latter process, thought to occur in the cells of colonic crypts [30], is the result of a cAMP- or Ca^{2+}-induced increase in apical membrane chloride conductance, followed by diffusive flux of chloride down its electrochemical gradient [16, 21]. The equilibrium potential of chloride in the secretory cells is maintained more positive than the apical membrane potential by virtue of the activity of a basolateral Na^+-K^+-$2Cl^-$ cotransport system and an increase in basolateral potassium conductance [16].

With respect to oxalate (and chloride) we found that: (1) basal oxalate absorption can be reversed to net secretion by dB-cAMP increases in J_{sm}^{Ox}, (2) dB-cAMP-stimulated secretory fluxes, but not basal absorptive fluxes, were fully blocked by serosal furosemide, and (3) the putative chloride channel blocker NPPB reduced J_{sm}^{Ox} about 35% of the stimulated flux.

The dB-cAMP reversal of net oxalate transport to secretion was due to persistent increases in J_{sm}^{Ox} and these changes were paralleled in magnitude by increases in J_{sm}^{Cl} and net chloride secretion. In some experiments, the increases in J_{sm} were accompanied by a small but significant depression of J_{ms}. This was most clearly seen in tissues that had been pretreated with furosemide when addition of secretagogue depressed the mucosal to serosal fluxes of oxalate and chloride and increased G_t without stimulating net secretion of either ion. These results suggest that dB-cAMP exhibits measurable antiabsorptive actions and decreases paracellular resistance as components of the suite of effects contributing to the secretory state in the rabbit distal colon.

The addition of furosemide to dB-cAMP-stimulated tissues fully blocked the ongoing secretion of oxalate and chloride by decreasing their serosal to mucosal components. Furthermore, furosemide pretreatment prevented the dB-cAMP-induced increases in both J_{sm}^{Ox} and J_{sm}^{Cl}, but was without effect on control fluxes. Taken together, these results suggests that the basolateral uptake of oxalate is mediated by the furosemide-sensitive Na^+-K^+-$2Cl^-$ cotransporter or by another, unidentified furosemide-sensitive mechanism.

The dB-cAMP-induced secretion of oxalate and chloride was substantially depressed (40%) by the addition of 5×10^{-5} M NPPB to the mucosal saline. This agent is generally considered to be a chloride channel blocker [4, 5, 27] and was employed in the present study with that expectation. In the rat distal colon, NPPB (10^{-4} M, mucosal) was reported to completely block forskolin-induced increases in J_{sm}^{Cl} and net chloride secretion [4]. However, in the latter study this drug also exhibited antiabsorptive effects on NaCl absorption, perhaps by interfering with the apical Cl^--HCO_3^- exchanger in this tissue. NPPB has also been shown to affect endogenous ATP levels in rabbit distal colon [17] and basolateral potassium channels in the bullfrog corneal epithelium

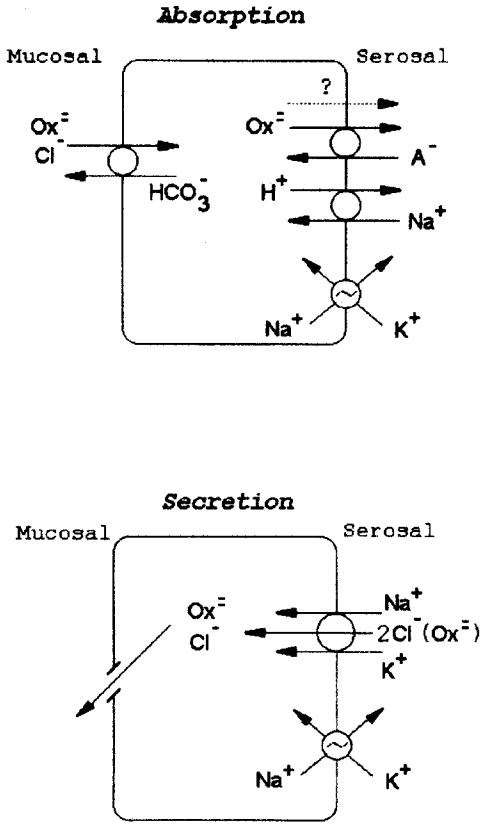

Fig. 1. Schematic summary of proposed mechanisms of transcellular oxalate transport across the rabbit distal colon. *Top panel* depicts net oxalate absorption: oxalate enters the enterocyte via a 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid-(SITS)-sensitive Cl^--HCO_3^- exchanger in the apical membrane [15]. Exit across the basolateral membrane is proposed to occur via a 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid-(DIDS)sensitive anion exchanger that is coupled to an amiloride-sensitive Na^+-H^+ antiporter. Additional efflux pathways for oxalate may exist as indicated by the *question mark*. The sodium pump maintains the Na^+ gradient. *Bottom panel* depicts net oxalate secretion: oxalate crosses the basolateral membrane and is accumulated intracellularly via a furosemide-sensitive (Na^+-K^+-2Cl^- cotransport?) pathway. Oxalate efflux from the secretory cell is hypothesized to occur via a 5-nitro-2-(3-phenylpropylamino)benzoic acid-(NNPB)-sensitive apical membrane pathway (Cl^- channels?)

when applied serosally [25]. In pilot studies we observed that 10^{-5} M NPPB frequently was without effect on secretory fluxes, whereas 10^{-4} M reduced secretion, but the higher concentration was often associated with a collapse of I_{sc} and G_t, indicating that the higher dose can have multiple effects in this tissue as noted by others employing higher concentrations [17]. Such ambiguities regarding the full extent of the action of NPPB can only be resolved by further experimentation. Nonetheless, under the conditions presented here, the most simple explanation for the actions of NPPB is that it reduces the activity of dB-cAMP-induced chloride channel activity thereby reducing the secretory flux of chloride. The fact that oxalate secretory fluxes were reduced in a quantitatively similar manner, suggests the possibility that oxalate efflux from the secretory cell is mediated via these chloride channels.

In conclusion, the distal colon of the rabbit exhibits the capacity to either absorb or secrete oxalate. As depicted in Fig. 1, the net absorptive flux of oxalate is proposed to be mediated by a SITS-sensitive, Cl^--HCO_3^- exchanger in the apical membrane of absorbing cells [15]. The basolateral exit of oxalate is distinct from the chloride exit mechanism. At least part of the efflux of oxalate (DIDS sensitive) is proposed to result from an anion exchange process that is indirectly coupled to a previously undescribed, amiloride-sensitive, Na^+-H^+ antiporter in the basolateral membranes of absorbing cells. Figure 1 also portrays a suggested mechanism that accounts for cAMP stimulation of oxalate secretion. Oxalate is transported across the basolateral membrane by a furosemide-sensitive (Na^+-K^+-2Cl^- cotransport?) pathway and is presumed to be accumulated intracellularly. Oxalate movement into the lumen is proposed to occur via a cAMP-induced, NPPB-sensitive pathway (chloride channel) by simple diffusion.

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