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OXALATE UPTAKE BY EVERTED SACS OF RAT COLON

REGIONAL DIFFERENCES AND THE EFFECTS OF pH AND RICINOLEIC ACID

SHELDON E. SCHWARTZ *, JOHN Q. STAUFFER, LLOYD W. BURGESS and MELISSA CHENEY

Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, NY 13210 (U.S.A.)

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Summary

Hyperoxaluria is a complication of disorders associated with steatorrhea. The colon is the presumed site of enhanced oxalate absorption in patients with steatorrhea. We performed studies of colonic mucosal oxalate uptake in everted sacs of rat colon to determine the kinetics of colonic oxalate transport and to evaluate the effect of both pH and ricinoleic acid, a hydroxy fatty acid, on colonic oxalate uptake. Our study demonstrated that oxalate is transported throughout the colon by passive diffusion. Tissue uptake increased linearly with increasing oxalate concentrations and was unaffected by metabolic inhibitors, oxygen deprivation, or temperature changes. There were pH-dependent regional differences of oxalate uptake both in the presence and absence of ricinoleic acid. In the absence of ricinoleic acid, the highest oxalate uptake occurred at the lower pH values (5.4 and 6.4). In the presence of ricinoleic acid oxalate uptake was enhanced at the higher pH values (7.4 and 8.4); a finding most likely related to decreased solubility of ricinoleic acid at pH 5.4 and 6.4. Intraluminal pH is an important determinant of colonic oxalate uptake in the presence and absence of ricinoleic acid.

Introduction

Hyperoxaluria is a complication of gastrointestinal disorders associated with steatorrhea [1—10]. Renal oxalate stones often complicate disorders associated

* To whom correspondence should be addressed.
Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid.

with hyperoxaluria (ileal inflammatory disease, ileal resection, and small bowel bypass procedures) [1—3,6,11—13]. The colon is the presumed site of enhanced oxalate absorption in patients with intestinal disease [3,5,8]. Patients with ileal disease, an intact colon, and steatorrhea develop hyperoxaluria while patients who have steatorrhea and have undergone ileal reseactions and total colectomy have normal urinary oxalate levels when stressed with high oxalate, low-calcium diets [5,7]. Perfusions of fatty acids and bile acids (independently or in combination) in rodents have demonstrated enhancement of oxalate absorption in the colon but no enhancement in the jejunum or ileum [14—16]; bile acid perfusions in the absence of fatty acids increase oxalate absorption in monkey and human colon [14,17].

Since the colon is the presumed site of enhanced oxalate absorption, characteristics of oxalate uptake were determined using everted sacs of rat colon. Oxalate uptake in proximal, middle and distal colon was determined at pH 5.4, 6.4, 7.4, and 8.4, in both the presence and absence of ricinoleic acid (12-hydroxy-*cis*-9-octadecanoic acid), a hydroxy fatty acid similar to those produced in excessive amounts by colonic bacteria in patients with steatorrhea [18—20]. Our results suggest that colonic absorption of oxalate is a passive process and that there are pH-dependent regional differences in colonic uptake of oxalate in both the presence and absence of ricinoleic acid.

Materials and Methods

Chemicals and reagents. Radiolabeled [^{14}C]oxalate and radiolabeled [^{3}H]-poly(ethylene glycol) 4000, both 99% pure as determined by chromatography, were obtained from New England Nuclear Corporation, Boston, MA, and used without further purification. Oxalate was obtained as the acid from J.T. Baker Chemical Company, Phillipsburg, NJ; *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer, 2,4-dinitrophenol, and ouabain from Sigma Chemical Company, St. Louis, MO; toluene and unlabeled poly(ethylene glycol) 4000 was obtained from Fisher Scientific Company, Fair Lawn, NJ; ricinoleic acid was obtained as the sodium salt from Eastman Kodak Company, Rochester, NY; and 2,5-diphenyloxazole (PPO), *p*-bis-2-(5-phenyloxazolyl)-1-benzene (POPOP) and NCS Tissue Solubilizer from Amersham/Searle Corporation, Arlington Heights, IL.

Solutions. Iced serosal, preincubation, and incubation solutions contained 10 mM Hepes buffer and 130 mM NaCl. In addition, incubation solutions contained unlabeled oxalate, 5 mM unlabeled poly(ethylene glycol) 4000, radiolabeled [^{14}C]oxalate (0.02 μCi/ml) and radiolabeled [^{3}H]poly(ethylene glycol) (0.04 μCi/ml). Poly(ethylene glycol) was used as a non-absorbable volume marker. Oxalate concentration was 5 mM except in kinetic studies where 0.25, 1 and 10 mM oxalate solutions were also prepared. Ricinoleic acid concentration was 8 mM. The pH was adjusted to the desired value using NaOH solution. Additional NaCl was added to adjust the osmolality to 290 ± 5 mosM/kg, as measured by freezing-point depression. In the kinetic studies, incubation solutions were modified to include 0.1 mM dinitrophenol or 0.1 mM ouabain. Liquid scintillation counting fluid contained 4 g of PPO and 50 mg of POPOP/l of toluene.

Gut sac preparation. Male Charles River rats (450—600 g) were maintained on an ad libitum normal rat chow and water diet. Stunned animals were decapitated and their abdomens were opened with a midline incision. The entire colon (from the cecal-colonic junction to the proximal rectum) was removed, flushed with cold 0.154 M NaCl, and stretched over a glass tube (8 mm diameter, 300 mm long). Fat was removed along the line of mesenteric attachment. The bowel was everted on the tube and the mucosal surface rinsed with Hepes buffer solution. Sacs were constructed by advancing the everted bowel partially off the glass tube, placing a 4-0 silk ligature at the distal end, filling the serosal compartment with unlabeled Hepes buffer solution through the glass tube, and finally placing a second 4-0 silk ligature 1.5—2 cm above the first. The sac was then cut from the remaining bowel and suspended in iced (4°C), oxygenated Hepes buffer at the appropriate pH and osmolality. Three sacs were constructed from each colon: one from the proximal portion (1—3 cm from the cecal-colonic junction), another from the midportion of the bowel (11—13 cm) and a third from the distal portion (22—24 cm). An average of 6—7 min elapsed between killing the animals and placing the sacs in iced unlabeled Hepes buffer. After 5 min in the iced unlabeled Hepes buffer, each sach was suspended for 3 min in a 37°C oxygenated preincubation unlabeled Hepes buffer. In each experiment the serosal and preincubation solutions were identical in composition and pH.

Flux rate determinations. Sacs were transferred from the preincubation solution and suspended for 10 min in a 37°C, oxygenated Hepes buffer solution containing both ^{14}C-labeled oxalate and ^{3}H-labeled poly(ethylene glycol). In each experiment the incubation solution was maintained at the same pH as the serosal and preincubation solutions. The duration of gut sac incubation in labeled Hepes buffer solution varied in those experiments which evaluated the effect of time of incubation on oxalate uptake. In the kinetic studies gut sac incubations were also performed at 25°C and in a nitrogen atmosphere. Following incubation, the sacs were removed, blotted, cut with a razor blade into two portions, and each portion transferred to tared vials, which were then dried overnight at 50°C. After cooling to room temperature, the vials were reweighed to determined dry tissue weight.

Dried tissue was rehydrated by the addition of 100 μl of distilled water and dissolved in 1 ml of NCS tissue solubilizer at 50°C. 15 ml of scintillation fluid was added and the samples counted on a Searle Mark III (Model 6880) liquid scintillation system using the variable quench dual-labeled isotope program for ^{3}H and ^{14}C. Net flux rates (nmol/100 mg dry tissue weight per min) were calculated using the technic of Sallee et al. [21]. The average of the two portions was used unless the values differed by more than 10% in which event the result was excluded from the data.

Corrections were then made for adherent mucosal fluid volume (μl/100 mg dry tissue weight), the volume of incubation fluid adherent to the tissue, and the amount of labeled oxalate present in the adherent mucosal fluid volume. This volume was calculated by dividing the amount of non-absorbable marker in the tissue sample by its concentration in the incubation medium. The amount of labeled oxalate present in the adherent mucosal fluid volume was subtracted from the amount in the tissue sample yielding the net oxalate flux.

Statistical analysis. All calculations were performed using the two-tailed Student's *t*-test (paired or unpaired samples as applicable). Data are expressed as mean net oxalate uptake ±1 S.E.

Results

Adherent mucosal fluid volume

During measurement of tissue uptake, fluid adherent to the tissue is carried over into the counting vial and may result in significant errors in the determination of uptake rates. To provide accurate correction for the adherent mucosal fluid volume samples were obtained at a time when the adherent mucosal fluid volume was labeled uniformly by the non-absorbable volume marker. [^{3}H]poly(ethylene glycol) achieved equilibrium within the first 5—8 min of incubation at pH 8.4 in all regions, both in the presence and absence of ricinoleic acid (Fig. 1). The adherent mucosal fluid volume increased in the presence of ricinoleic acid at all four pH values; the effect of ricinoleic acid was greatest at pH 7.4 and 8.4 (Fig. 2). The stable adherent mucosal fluid volume measurement after 6 min and the work of others [22] support the validity of poly(ethylene glycol) 4000 as a suitable extracellular fluid marker and exclude the possibility that the increase in adherent volume seen in the presence of ricinoleic acid is caused by alterations in mucosal permeability which allow poly(ethylene glycol) 4000 to diffuse into the cell (this increase in adherent mucosal fluid volume has also been described with bile salt exposure) [23].

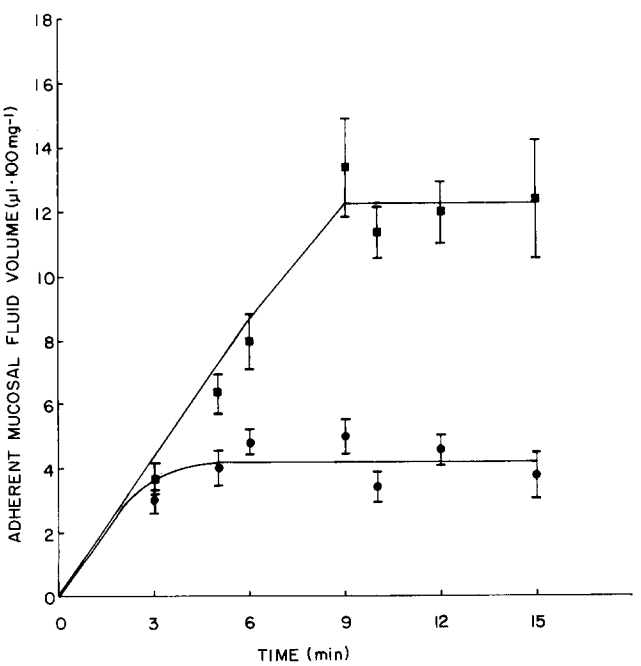

Fig. 1. Adherent mucosal fluid volume during distal colonic segment sac incubation in labeled Hepes buffer containing 5 mM oxalate at pH 8.4 in the presence (■) and absence (●) of ricinoleic acid. Each point represents mean adherent mucosal fluid volume (µl/100 mg dried tissue weight) ±1 S.E. for determinations in 6—10 animals.

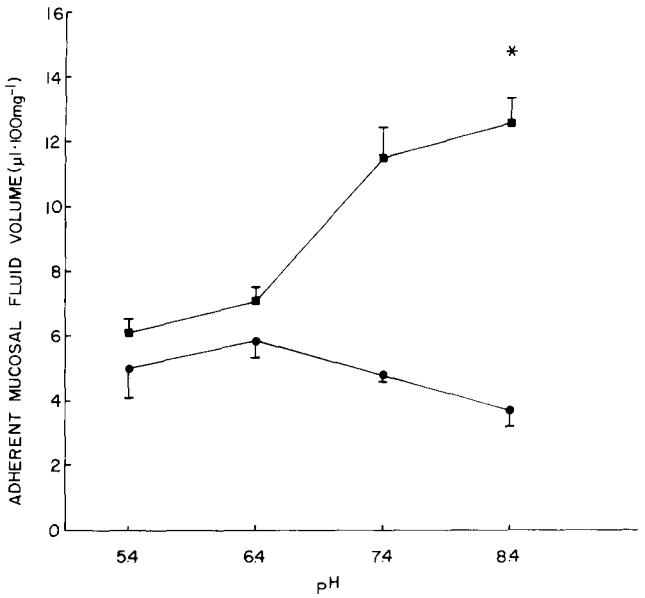

Fig. 2. Adherent mucosal fluid volumes after a 10 min incubation in Hepes buffer containing 5 mM oxalate in the presence (■) and absence (●) of ricinoleic acid at varying pH levels. Each point represents mean adherent mucosal fluid volume (µl/100 mg dried tissue weight) ±1 S.E. for determinations in 7—9 animals. The mean adherent mucosal fluid volume is the mean of all three segments. * $P < 0.001$ for comparison of mean adherent mucosal volume at a given pH level.

Characteristics of mucosal oxalate uptake

Oxalate uptake was linear with respect to time beyond 6 min of incubation and extrapolated to near zero at zero time at pH 5.4, 6.4, 7.4, and 8.4. Fig. 3 depicts mucosal uptake of oxalate at pH 8.4; this is representative of time vs. uptake data at other pH levels. (Uptake of oxalate measured during incubation periods of less than 6 min was elevated; there was incomplete equilibration of the volume marker within the adherent mucosal fluid volume at these incubation periods.)

Tissue oxalate uptake increased linearly in all regions with increasing oxalate concentrations at pH 5.4 and 8.4. The data exclude saturation kinetics in the concentration range of 0.25—10 mM oxalate (Fig. 4). Uptake was unaffected by discontinuing oxygenation and saturating the buffer with nitrogen, decreasing incubation temperature to 25°C, or incubating colon sacs in buffer to which 0.1 mM dinitrophenol or 0.1 mM ouabain was added (Table I). The kinetics exhibited throughout the colon were those of a passive transport system, i.e. energy independent and non-saturable. No calculations were made to account for distortion of net flux by the unstirred water layer. Oxalate is extremely water soluble in buffer free of divalent cations. The passive diffusion of a highly water-soluble molecule is most dependent on the rate of cell membrane penetration. The effect of the unstirred water layer on oxalate absorp-

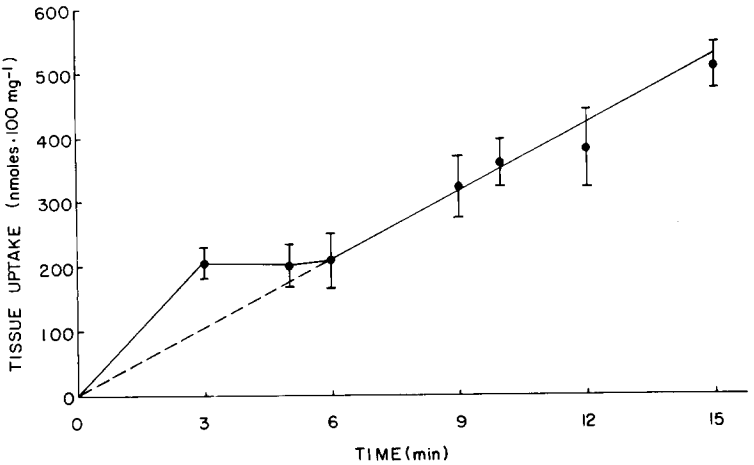

Fig. 3. Mean oxalate tissue uptake during 15 min time course. Proximal colonic segment sacs were incubated in labeled Hepes buffer containing 5 mM oxalate at pH 8.4. Uptake is linear from 6 to 15 min. ------, extrapolation of data from 0 to 6 min. Each point represents the mean mucosal uptake (nmol/100 mg dried tissue weight) ±1 S.E. for determinations in six animals.

tion would be expected to be relatively minimal.

An incubation period of 10 min was chosen for subsequent experiments. At 10 min the uptake of oxalate was linear in relationship to time, and the adherent mucosal fluid volume was uniformly labeled by the volume marker. Serosal fluid samples after 10 min of incubation showed only background counts.

Regional oxalate uptake within the colon and the effect of differing pH values

Uptake of oxalate by colonic mucosa varied regionally within the colon. Proximal colon absorbed oxalate more rapidly than distal colon at pH 6.4, 7.4, and 8.4 ($P < 0.025$, $P < 0.025$, $P < 0.001$). At pH 7.4 and 8.4 uptake of oxalate in the proximal segment was also higher than in the middle segment ($P < 0.05$, $P < 0.025$). Mucosal oxalate uptake at pH 5.4 was uniform in all segments (Table II and Fig. 5).

Oxalate flux varied as the pH changed in the middle and distal, but not in the proximal colonic segments. Oxalate flux in the middle segment was lower at pH 5.4 than at pH 7.4 and 8.4 ($P < 0.05$, $P < 0.025$). Oxalate flux rate in the distal segment was lower at pH 8.4 than at pH 5.4, 6.4, and 7.4 ($P < 0.005$, $P < 0.025$, $P < 0.025$) and higher at pH 5.4 than at pH 6.4, 7.4, and 8.4 ($P < 0.05$, $P < 0.05$, $P < 0.005$).

Effect of ricinoleic acid on oxalate uptake at differing pH values

Oxalate uptake increased in the presence of ricinoleic acid in the proximal, middle and distal segments at pH values 7.4 and 8.4 when compared to the flux rates in the absence of ricinoleic acid ($P < 0.005$, $P < 0.01$) (Table II and Fig. 5). No statistically significant increase in oxalate uptake was noted at pH values 5.4 and 6.4. The incubation solutions were turbid at pH 5.4 and 6.4 and an oily layer was noted on the surface at these pH values.

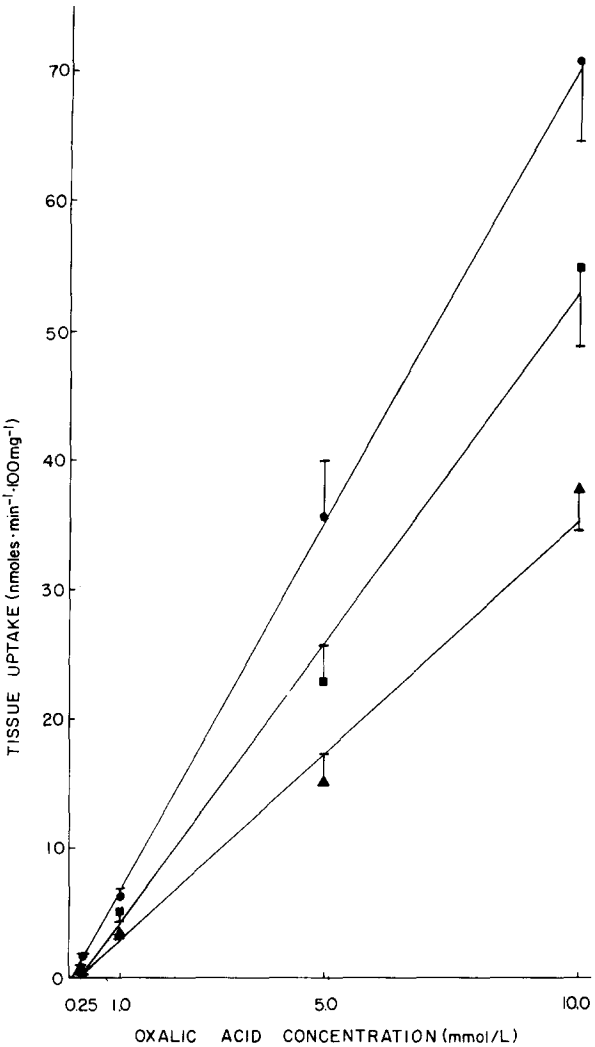

Fig. 4. Regression lines of tissue uptake of oxalate in proximal (●), middle (■), and distal (▲) colonic segments at different oxalate concentrations. Colonic sacs were incubated for 10 min in labeled Hepes buffer maintained at pH 8.4 with oxalate concentrations of 0.25, 1, 5, and 10 mM. Each point represents the mean mucosal uptake (nmol/100 mg dried tissue weight/min) ±1 S.E. for determinations in six animals.

TABLE I

DETERMINATION OF TISSUE UPTAKE CHARACTERISTICS

Mucosal uptake of oxalate in everted sacs of rat colon. The sacs were incubated for 10 min in labeled Hepes buffer containing 5 mM oxalate at pH 8.4. Data expressed as mean mucosal uptake (nmol/100 mg dry tissue weight/min) ± 1 S.E. for four everted sacs of rat colon.

	Mucosal uptake		
	Proximal segment (1—3 cm)	Middle segment (11—13 cm)	Distal segment (22—24 cm)
37°C	37.4 ± 8.1	22.7 ± 6.7	14.1 ± 4.4
25°C	31.9 ± 10.6	25.4 ± 6.1	12.8 ± 5.2
0.1 mM dinitrophenol	33.9 ± 10.0	20.9 ± 3.2	13.7 ± 4.4
0.1 mM ouabain	30.7 ± 3.9	18.3 ± 7.3	15.2 ± 7.3
Nitrogen atmosphere	34.7 ± 8.9	24.6 ± 7.3	16.6 ± 2.2

TABLE II

REGIONAL OXALATE UPTAKE IN THE PRESENCE AND ABSENCE OF RICINOLEIC ACID (RA) AT VARYING pH LEVELS

Mucosal uptake of oxalate in everted sacs of rat colon. The sacs were incubated for 10 min in labeled Hepes buffer containing 5 mM oxalate. Data expressed as mean mucosal uptake (nmol/100 mg dried tissue weight/min) ± 1 S.E. (n). Number of individual determinations in parentheses.

pH	Proximal segment (1—3 cm)		Middle segment (11—13 cm)		Distal segment (22—24 cm)	
	Basal	RA	Basal	RA	Basal	RA
5.4	38.5 ± 3.1 (7)	42.6 ± 5.9 (7)	42.7 ± 7.6 (8)	29.6 ± 9.8 (6)	43.0 ± 0.9 (7)	34.9 ± 4.8 (7)
6.4	34.6 ± 4.2 (9)	48.2 ± 5.2 (8)	31.5 ± 4.0 (9)	29.8 ± 4.0 (7)	23.8 ± 3.2 (10)	27.8 ± 4.7 (8)
7.4	33.5 ± 3.0 (8)	54.6 ± 5.7 (8)	22.7 ± 3.6 (9)	57.5 ± 4.1 (8)	22.8 ± 2.5 (8)	43.6 ± 3.4 (7)
8.4	34.9 ± 3.1 (8)	49.3 ± 4.5 (9)	22.4 ± 2.4 (8)	42.5 ± 3.6 (9)	13.5 ± 2.0 (8)	47.2 ± 4.1 (9)

Within the middle and distal segments oxalate flux, in the presence of ricinoleic acid, varied with changes in pH. In the middle segment oxalate flux at pH 5.4 was lower than the flux at pH 7.4 ($P < 0.025$) and the flux at pH 6.4 was lower than the flux at pH 7.4 and 8.4 ($P < 0.001$, $P < 0.05$). In the distal segment oxalate flux was lower at pH 6.4 than the flux at both pH 7.4 and pH 8.4 ($P < 0.025$, $P < 0.025$).

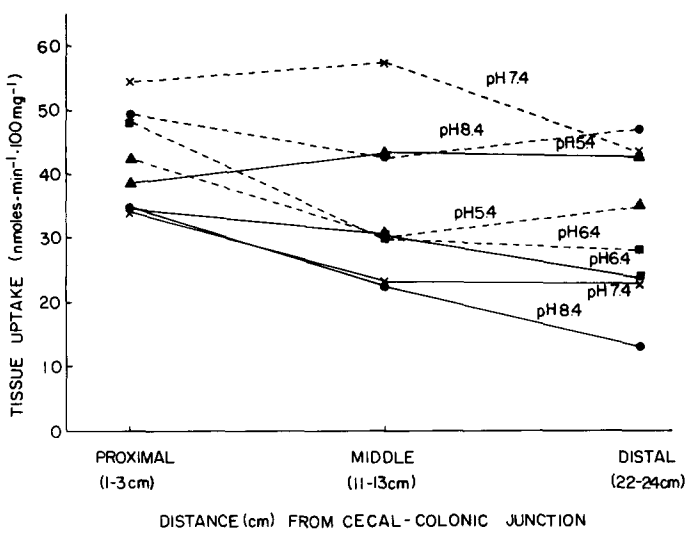

Fig. 5. Tissue uptake of oxalate in the absence (———) and the presence (- - - - -) of ricinoleic acid in proximal, middle, and distal colonic segments at pH 5.4 (▲), 6.4 (■), 7.4 (X), and 8.4 (●). Colonic sacs were incubated for 10 min in labeled Hepes buffer containing 5 mM oxalate. Each point represents the mean mucosal uptake (nmol/100 mg dried tissue weight/min) determinations in 5—10 animals.

Discussion

Our studies demonstrate that: (1) oxalate is transported by a passive diffusion process throughout the rat colon; (2) there are regional differences in oxalate uptake, and (3) pH affects oxalate absorption both in the presence and absence of ricinoleic acid.

Binder found that oxalate was not transported in colon in the absence of a concentration gradient [24] and concluded that colonic oxalate transport was a passive process. In our study colonic oxalate uptake increased with increasing oxalate concentrations, and oxalate flux was unaffected by oxygen deprivation, metabolic inhibition, $(Na^+ + K^+)$-ATPase inhibition or temperature modifications. Our data when coupled with the work of Binder [24] demonstrate that oxalate is transported throught the colon by an energy-independent, nonsaturable process. Factors which alter intraluminal oxalate solubility should affect oxalate absorption. A low-velocity, energy-independent, saturable process with a significant passive component cannot be excluded. In such a situation the effect of metabolic inhibitors would be extremely slight and would be enveloped in our system's error. We do not know, however, of any nutrient which is passively transported in small bowel and actively transported in colon.

Regional differences in solute transport (other than electrolytes) have not been demonstrated in the colon. At pH values 6.4, 7.4, and 8.4 the ability of the colon to absorb oxalate diminishes distally. Surface area alone cannot explain differences in oxalate flux. Firstly, at pH 5.4 oxalate uptake is uniform throughout the colon, and secondly, within a given region the uptake of oxalate varies as the pH changes. If alterations in surface area alone account for diminished oxalate uptake in the distal colon, then the ratio of proximal uptake to distal uptake at all pH levels should be identical. Marked differences in these ratios are evident. Oxalate absorption in the middle and distal segments is affected by changes in pH. This finding cannot be explained by the pK of oxalate ($pK_1 = 1.4$ and $pK_2 = 4.3$). At the four pH values virtually all of the oxalate is present as a divalent anion. On the basis of ionization alone, oxalate uptake should be identical at these four pH values. Changes in pH might affect the mucosal membrane by altering the charges of the membrane proteins and influence anion fluxes. A divalent anion will be dramatically influenced by alterations in the electrical profile of the epithelium.

Non-absorbed fatty acids may augment intestinal oxalate absorption by binding calcium originally bound to oxalate, therefore, increasing concentrations of soluble oxalate [4,10,14], by altering the permeability of the intestinal mucosal membrane [16,22,25—27] or by being metabolized by colonic bacteria into hydroxy fatty acids which then alter mucosal permeability [25]. Our study demonstrates that while increased oxalate absorption occurs in the presence of ricinoleic acid, the pH of the buffer solution is critical. Ricinoleic acid affects oxalate uptake when in solution and pH is a determining factor in the solubility of ricinoleic acid.

Intraluminal pH and regional transport differences are important factors affecting colonic oxalate absorption, in addition to intraluminal calcium, oxalate, bile salt, and fatty acid concentrations. pH values in this study were chosen to encompass the range seen in the colon and distal ileum of normal

individuals [28]. Previously reported differences in urinary oxalate excretion in jejunoileal bypass subjects as opposed to patients with regional enteritis and a right hemicolectomy and ileectomy may be in part attributed to loss of the proximal colon and/or differences in intraluminal pH. In diseases associated with significant steatorrhea, unabsorbed fatty acids may exceed the concentration of ricinoleic acid used in our studies [29]. If ricinoleic acid or other hydroxy fatty acids, e.g. 10-hydroxystearic acid (10-hydroxyoctadecanoic acid) are present in appreciable concentration in the colon, intraluminal pH may dictate the degree of hyperoxaluria [19,30].

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