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INVITED REVIEW

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Intestinal transport of an obdurate anion: oxalate

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Abstract In this review, we focus on the role of gastrointestinal transport of oxalate primarily from a contemporary physiological standpoint with an emphasis on those aspects that we believe may be most important in efforts to mitigate the untoward effects of oxalate. Included in this review is a general discussion of intestinal solute transport as it relates to oxalate, considering cellular and paracellular avenues, the transport mechanisms, and the molecular identities of oxalate transporters. In addition, we review the role of the intestine in oxalate disease states and various factors affecting oxalate absorption

Keywords Absorption · Secretion · Anion exchange · Renal · Dietary

Introduction

For patients with oxalate associated diseases and researchers interested these disease states, oxalate is indeed an obdurate anion. It is usually present in minute amounts relative to other anions, yet small changes in its concentration in the presence of calcium can lead to calcium oxalate deposition. Historically, it has been very challenging to analytically quantitate. It can exist as a monovalent or divalent anion. It is not metabolized in humans, but is readily generated by a number of metabolic pathways. Its chief mischief is caused by its partnership with calcium, which not only necessitates costly medical management, but also renders it tricky to study in many experimental settings. Because plasma oxalate concentrations are dependent upon dietary load, intestinal absorption, metabolic production, and renal excretion, research in this arena encompasses a wide variety of disciplines. In this review, we focus on the role of gastrointestinal oxalate transport primarily from a contemporary physiological standpoint with an emphasis on those aspects that we believe may be most important in efforts to mitigate the untoward effects of the oxalate anion.

We begin with a general discussion of intestinal solute transport as it relates to oxalate, considering cellular and paracellular avenues, their reported mechanisms, and the molecular identities of oxalate transporters. Subsequent sections consider the role of the intestine in oxalate disease states and various organic, inorganic, and microbial factors affecting oxalate absorption. Previous reviews of oxalate transport in intestinal [1, 2] and renal epithelia [3] may be consulted for additional perspectives and studies not addressed here.

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Gastrointestinal oxalate transport mechanism

In the following discussion, the transepithelial movement of oxalate will be considered from a mechanistic standpoint. This necessarily involves considerations of the available routes for oxalate flux in traversing the epithelial barrier (cellular and paracellular pathways) and the specific possible mediators (anion exchangers, etc.) for this translocation process at the two membrane surfaces.

For any solute moving across an intestinal segment there are two morphologically distinct routes which may be envisioned: movement between epithelial cells (paracellular or shunt pathways) and/or movement through the cells (transcellular pathways) comprising the epithelial barrier. Paracellular solute flux occurs as a consequence of the properties of junctional assemblies of proteins that collar the apical pole of the enterocytes providing both mechanical and functional coupling of

neighboring cells [4]. Transcellular solute flux involves movement across two separate plasma membrane barriers: the apical (mucosal) membrane and the basolateral (serosal) membrane. These cellular membrane barriers are arranged in series with one another and are uniquely different in their composition and function by virtue of pole-specific targeting and insertion of various membrane proteins. In the sense of an equivalent circuit, the transcellular barriers are arranged in series with one another and are in parallel with the paracellular pathway—hence, solute flow is determined by the permeability of both parallel avenues. The relative contribution of these pathways to the unidirectional flux of a solute is variable along the length of the intestine and is frequently related to the magnitude of electrical resistance of the paracellular pathway. Since the cellular elements offer, for the most part, a relatively constant electrical resistance from one intestinal segment to another, the ''leakiness'' or ''tightness'' of a particular intestinal segment is determined by the permeability of paracellular junctional pathways [4]. (This has considerable significance to the transepithelial flux of small solutes like oxalate, as will be described later.) In contrast, transcellular electrolyte transport is usually mediated and can lead to vectorial transport (net absorption or secretion) that is, at least, indirectly coupled to energy-dependent processes (i.e., active transport). It is important to recall that any phenomenological quantitation of the magnitude of oxalate flow, be it a unidirectional flux measured in vitro or intestinal absorption estimated from in vivo experiments, necessarily involves both cellular and paracellular avenues.

Transport of a solute from the mucosal to the serosal side of an epithelium through cellular and paracellular pathways is frequently referred to as an absorptive flux (symbolized as J_{ms}) and is given in terms of moles of solute translocated per unit time per unit area or mass. Solute may also flow from the serosal to mucosal side of the tissue giving rise to another quantifiable unidirectional flux (again possibly via cellular and paracellular pathways, symbolized J_{sm}). For some analyses the net flux ($J_{net} = J_{ms} - J_{sm}$) is the principal variate of interest because it portends the active, transcellular nature of the transport mechanisms involved.

Paracellular transport

Flow of oxalate through the paracellular pathway (or shunt) is passive, driven by the prevailing electrochemical gradient across the epithelium and conforms to free solution diffusion with restrictions imposed by junctional elements that may sterically and/or electrostatically retard the diffusion process. In the absence of electrochemical gradients (as in Ussing type experiments) the paracellular flux of oxalate in the M to S direction is equal to the paracellular flux of oxalate in the S to M direction. The situation in vivo is markedly different as there are both electrical and concentration gradients of oxalate across intestinal epithelia. For example, the small intestinal lumen is frequently negative with respect to the serosal side due to active sodium absorption, and luminal free oxalate might be expected to be greater than plasma oxalate concentration, especially along the early segments. Both of these forces act to drive passive oxalate absorption through paracellular pathways in proportion to their magnitudes and the ''leakiness'' of the segment. From these considerations it is expected that the contribution of paracellular flux of oxalate to net oxalate absorption in vivo is significant, however, this simple expectation remains to be verified systematically along intestinal segments. As discussed in a subsequent section regarding the importance of bile and fatty acids in enteric hyperoxaluria, the paracellular avenue for oxalate absorption may become the dominant path.

It is somewhat easier to evaluate the importance of paracellular pathways using isolated segments of intestine in conventional Ussing-type chambers. When the electrochemical gradients are nullified, any net oxalate transport in either the absorptive or secretory direction, measured with ^{14}C-oxalate as a tracer, largely reflects active, transcellular, mediated transport of the oxalate anion. Consequently, the back flux of oxalate is an approximation of flow through the shunt pathway and, in general, the unidirectional back fluxes of oxalate are as large, or larger than, the calculated net flux for any intestinal segment. For example, oxalate back flux through the shunt path is 76% of J_{net}^{Ox} in rat ileum, 133% in rat colon [5], 400% in rabbit proximal colon [6], and 110% in rabbit distal colon [7]. Clearly, the shunt path can contribute significantly to oxalate exchanges along the mammalian intestine, but, as noted above, this remains to be systematically quantitated in vivo.

Transcellular transport

Transport of any solute across a cell membrane may be mediated by integral membrane proteins (carriers and channels), as is the case for hydrophilic compounds and electrolytes, whereas the transmembrane flux of hydrophobic solutes may not necessitate a special carrier to partition into the membrane (non-mediated).

Non-mediated transport

Weak acids can permeate an epithelial barrier, or at least gain entry to the cytoplasm of an enterocyte, by non-ionic diffusion if the luminal pH is sufficiently low to fully protonate the anion. Transport by non-ionic diffusion is a passive, non-mediated, gradient driven process that is a consequence of the increased lipid solubility or oil/water partition coefficient of the protonated species. Once inside the cytoplasmic compartment weak acids ionize, which functionally traps the solute in the cell where it may move across the basolateral membrane by a mediated mechanism(s). In the case of oxalate,

which is a relatively strong acid ($pK_1 = 1.2$, $pK_2 = 4.2$) the only segment of the gastrointestinal tract that exhibits a bulk hydrogen ion activity that would be required to protonate the oxalate anion is the stomach. In the gastric compartment oxalate flux through the transcellular pathway could be enhanced because in an acidic environment the overall permeability coefficient of oxalic acid is expected to be much higher than that of either the mono- or divalent anion. A higher lipid solubility of non-ionized oxalate favors simple gradient-driven partition into the lipid barriers of the apical membrane as a first step in the transcellular translocation process. For such a mechanism to be meaningful to trans-gastric oxalate absorption there must be an avenue for oxalate anion efflux across the basolateral membrane.

Oxalate absorption by the stomach, while potentially significant, has received scant attention experimentally. The most recent study compared urinary oxalate excretion following an oxalate load (spinach) in healthy adults compared with patients having total gastrectomy [8]. Urinary oxalate excretion in the healthy individuals peaked at 40 min and again at 3 h after the load. In the gastrectomy patients, however, the first peak was absent, indicating a contribution of the gastric mucosa to oxalate absorption along the alimentary tract. In an earlier study, urinary excretion of oxalate was examined in adults following gastric administration of 5 mmol oxalate loads [9] where gastric emptying was blocked by an intrapyloric balloon. This study showed that with increasing gastric loading time there was a linear increase in urinary oxalate excretion and after 6 h over 60% of the load was absorbed. Both of these studies suggest that oxalate absorption by the stomach can be significant and presumably occurs by way of non-ionic diffusion of the free acid as noted above. We presume this is a transcellular phenomenon because gastric epithelia are usually among the ''tightest'' of intestinal barriers [10] (a useful feature which presumably minimizes back diffusion of actively secreted protons) so that oxalic acid diffusion through paracellular pathways in the stomach is probably of minor significance. It should be noted that there have been no studies directed at characterizing other transport pathways for oxalate in the various regions of the stomach, and this includes basolateral exit mechanisms for oxalate anion accumulating in gastric cells by non-ionic diffusion.

The contribution of non-ionic diffusion to oxalate absorption is generally viewed as a gastric phenomenon. However, any enterocyte that develops an acidic microclimate in the unstirred layer adjacent to the enterocyte is theoretically capable of supporting oxalate uptake by non ionic-diffusion. Also, there may be pathological alterations in luminal pH that would favor elevated oxalate absorption by non-ionic diffusion. For example, it has been shown that acidification of the caecum in lactulose-induced chronic colonic acidification in rats enhances intestinal oxalate absorption and may contribute to hyperoxaluria [11].

Mediated transport

Transepithelial transport of the oxalate anion through the cellular pathway is mediated at both poles of the enterocyte and is of considerable importance and interest because these avenues provide the potential for movement against the prevailing electrochemical gradient (secondarily active) and afford possible regulatory features not available for paracellular or non-mediated transcellular passive transport. We have shown that active, transcellular oxalate transport can proceed in either absorptive or secretory directions in rats and rabbits [5, 6, 7, 12] and is generally segment specific. Thus, the typical handling pattern varies from spontaneous net secretion in the small intestine to net absorption in the distal colon in healthy animals that we have examined under short-circuiting conditions [5, 7]. Not only does segmental heterogeneity exist with respect to oxalate handling, there is considerable evidence suggesting that these patterns are regulated by neuro-hormonal input and their respective cellular messengers, as outlined in the following sections.

Absorption Initially, oxalate absorption by the mammalian intestine was viewed as a passive, purely gradient-driven, non-mediated process presumably through shunt pathways [13, 14, 15]. These conclusions were based on experiments performed with low Ca^{2+} buffers (to mitigate the formation of CaOx in luminal media—the obdurate anion again) when Ox^{2-} concentrations were increased experimentally; but such maneuvers also promoted a decrease in tight junction resistance leading to a large increase in paracellular oxalate flux [16]. Net transcellular absorption of oxalate was first established in vitro using rat large intestine [16], and subsequently a number of studies employing both large and small intestinal segments have verified the existence of transcellular routes for oxalate transport. As noted above, transcellular oxalate flux involves an apical membrane uptake processes followed by efflux from the enterocyte across the basolateral membrane .

In rabbit distal colon, net oxalate absorption and J_{ms}^{Ox} were sensitive to metabolic and transport inhibitors, which suggested that the absorptive process was a mediated, energy-dependent process [17]. Furthermore, inhibition of carbonic anhydrase activity with acetazolamide in bicarbonate-free media blocked oxalate and chloride absorption while mucosal addition of the stilbenes (SITS) 10^{-4} M abolished the net flux of both oxalate and chloride [7, 17]. These features suggested that the apical entry process was secondarily active and perhaps mediated by a Cl^-/Ox^{2-}-HCO_3^- exchange mechanism. The possibility that oxalate uptake from the mucosal environment by an ion exchange mechanism was also supported by later studies using rabbit ileal brush border membrane vesicles documenting Cl^--Ox^{2-} and Ox^2-OH^- exchange processes [18].

The mechanism of oxalate efflux across the basolateral membrane into the serosal compartment during

absorption by rabbit distal colon appears also to be mediated by an anion exchange process as well, but one that does not necessarily involve chloride. In this tissue, J_{net}^{Ox} was inhibited 43% by low concentrations of serosal DIDS (10^{-6} M) without affecting net Cl^- absorption. Interestingly, serosal inhibition of Na^+-H^+ exchange with amiloride or dimethyl amiloride (1 mM) or using sodium-free serosal buffers blocked net oxalate absorption by reducing J_{ms}^{Ox}, implying that some portion of the basolateral efflux of oxalate is mediated by an ion exchange process that is coupled to intracellular pH by a Na^+-H^+ exchange antiporter [6]. As with events at the apical membrane, studies employing isolated basolateral membrane vesicles from the rabbit ileum suggest the existence of several flavors of anion exchange systems that are capable of mediating oxalate exchange in that tissue [19].

Based on these and other observations, we proposed that transcellular oxalate absorption is mediated apically by an Ox^{2-} (Cl^-)-HCO_3^- exchanger and basolaterally by other exchangers—at least one of which is possibly involved in base exchange across the basolateral membrane. These studies also highlight an important feature of oxalate transport by epithelia—namely, that by being involved with exchangers that translocate base, the mechanisms supporting oxalate absorption in the intestine are not likely to be simple and may be indirectly coupled to a variety of transporters that are involved with acid-base regulation of the enterocyte.

Secretion As noted previously, not only does the mammalian intestine absorb oxalate by energy-dependent transcellular pathways, it also exhibits the ability to support net, transcellular oxalate secretion under short-circuiting conditions in a segment-specific manner. In general, small intestinal segments [5, 18] and proximal colon [6] spontaneously secrete oxalate, while distal colonic segments from healthy animals actively absorb oxalate [7, 20]. Oxalate secretion by isolated intestinal segments, either spontaneously or in response to secretagogues, has an impact on how we view oxalate handling by the intestine from both mechanistic and functional standpoints. Functionally, net oxalate secretion across the intestine can serve as an extra-renal avenue for oxalate excretion (see below), and mechanistically, this phenomenon adds another interesting, albeit complicated, layer to be unraveled and distinguished from absorptive mechanisms.

Mechanisms of transepithelial oxalate secretion have been examined, most profitably, in the short-circuited colon following stimulation by secretagogues like cAMP analogues. At the present time, we can generally say that cAMP-mediated oxalate secretion resembles cAMP-stimulated chloride secretion: both processes are stimulated by cAMP [6, 17, 21], at least partly blocked by serosal loop diuretics like furosemide [17], partially inhibited by low concentrations of a chloride channel blocker NPPB [21] from the mucosal side, and occur as a result of both an increase in J_{SM} and a decrease in J_{MS}. As with transcellular absorption, ion-substitution studies suggest the oxalate secretory process is markedly dependent on other ions. For example, in a spontaneously secreting tissue like the rabbit proximal colon [6], removal of Na^+ abolishes net secretion; removal of Cl^- significantly inhibits secretion; while HCO_3^- removal promotes a vigorous net absorption of oxalate exclusively through a decrease in J_{sm}^{Ox}.

It has been proposed that secretagogue-stimulated oxalate secretion by rabbit distal colon occurs as follows: basolateral uptake and intracellular accumulation of oxalate anion by a loop diuretic-sensitive uptake mechanism followed by passive diffusion of accumulated oxalate across the apical membrane through a channel-like pathway [7]. The proposed apical membrane events are supported by studies employing rabbit ileal brush border membrane vesicles which revealed an electrogenic oxalate transport pathway that was poorly saturable, had a low activation energy, and exhibited kinetic and thermodynamic characteristics that suggest permeation through a channel-like mechanism like cystic fibrosis transmembrane conductance regulator (CFTR) regulated chloride channels [21]. Such findings and proposals do not exclude anion exchange mechanisms for oxalate secretion; indeed, it has been recently shown that a number of the SLC26 anion exchangers have regulatory components (PDZ domains in the COOH terminus) that may provide cAMP-sensitivity through CFTR [22, 23, 24, 25].

Regulation of transcellular oxalate transport

Both transcellular oxalate absorption and secretion exhibit strong dependencies on other ions like Na^+, Cl^- and HCO_3^-. Transport pathways involving these ions are strongly regulated in intestinal epithelia by a variety of neuro-hormonal agents because they are the principal osmolytes of plasma and are involved in the homeostatic control of cellular and systemic acid-base status. Because oxalate transport is coupled to the movements of these ions, it is expected that oxalate absorption and secretion may be affected by these same neuro-hormonal agents; however, there is relatively little information concerning the regulation of intestinal oxalate transport. Certainly, there is ample evidence that oxalate secretion by the distal colon can be promoted by cAMP-dependent pathways which also stimulate Cl^- and HCO_3^- secretion, and we have reported the importance of angiotensin II as a mediator/regulator of oxalate secretion in chronic renal failure (see below). In the rabbit proximal colon, which normally secretes oxalate, addition of epinephrine (50 μM, serosal) dramatically alters net oxalate transport: from a net secretion (−18 pmoles cm^{-2} hr^{-1}) to net absorption (+21 pmoles cm^{-2} hr^{-1}) by an increase in J_{ms}^{Ox} and a larger decrease in J_{sm}^{Ox} [6].

Since both magnitude and direction of net transepithelial oxalate transport can be manipulated by agents

promoting absorption and/or secretion, these regulatory aspects warrant further attention.

Molecular identification of anion exchangers

As noted above, anion exchange has been an expected feature of oxalate transport mechanisms in intestinal epithelia since the initial observations of stilbene (SITS and DIDS) sensitivity, carbonic anhydrase sensitivity, and dependence on extracellular Cl^- and HCO_3^- [17]. At the time of these investigations the molecular identity of these transport mechanisms in intestinal epithelia was not established. While it is still not possible to describe the individual exchange systems involved in transepithelial oxalate transport in the intestine, considerable progress towards that goal has been achieved recently by molecular cloning and expression studies of gene families that code for a variety of anion exchangers [26, 27]. In the following section, we outline these findings as they relate to intestinal epithelia in general and their potential for providing a better understanding of transcellular oxalate transport by these tissues.

The solute carrier gene family SLC26 encodes at least ten anion exchangers which may be generally characterized by the variety of mono- and divalent substrates they employ and their tissue distribution [26]; hence, their physiological significance is likely to be diverse. Several of these genes are expressed in intestinal tissues and accept oxalate as a substrate; requisite critical elements for transcellular oxalate transport by anion exchange. The SLC26A3 gene product, also known as down regulated in adenoma (DRA) has been shown to be present in human duodenum, ileum, caecum, distal colon, but not in the esophagus or stomach [28]. It is also found in the duodenum and colon of rats and rabbits [29], and in rats DRA mRNA it is equally abundant in proximal and distal colon [30]. In mice, DRA was highly expressed in caecum, proximal colon, and distal colon and less in the small intestine [31], and in another study human DRA mRNA was present in distal ileum and proximal colon [32]. Where attempts have been made to identify the cellular location of the DRA protein using immunohistochemistry, it appears to be associated with the apical pole of the enterocytes [28, 29]. Heterologous expression of DRA protein in *Xenopus* oocytes suggests a major function as a sodium-independent, DIDS sensitive, Cl^--base exchanger [33, 34, 35], and other studies suggest divalent anions like SO_4^{2-} and oxalate may also act as substrates [29, 36, 37], albeit with lower affinities. DRA mRNA is also expressed Caco-2 cells [38, 39], where it has been reported to mediate DIDS sensitive, SO_4^{2}-OH^- exchange which was competitively inhibited by oxalate and chloride ions [39]. An oxalate sensitive SO_4^{2}-OH^- exchange in human proximal colonic apical membrane vesicles has also been reported, which may be DRA-mediated [32].

While the importance of the DRA protein to apical membrane oxalate exchange in enterocytes is somewhat arguable and needs to be more fully explored, it is generally agreed that the SLC26A6 gene which expresses putative anion transporter-1 (PAT-1) is an excellent candidate oxalate transporter [26]. Much of the information regarding this gene and its product has been obtained from the renal proximal tubule where it has been suggested to represent the apical membrane Cl^--formate (oxalate) exchanger (hence the alias CFEX). In mice there is abundant expression of PAT-1 mRNA in stomach and in all segments of the small intestine, but only weak expression in the large intestine [33, 40], a pattern that is opposite that for DRA. However, in commercial human cDNA panels, SLC26A6 was expressed in colon and to a smaller degree in the small intestine [24]. In the mouse duodenum it appeared to be localized to villous (not crypt) cell apical membranes [33] and apical membranes of parietal cells of the gastric mucosa [41] as in the proximal tubule [42, 43, 44, 45]. Interestingly, in pancreatic duct cells the PAT-1 protein is located in the apical membrane and is increased in cells expressing functional CFTR as is DRA, and both are decreased in cells lacking functional CFTR [25]. Mouse PAT-1 expressed in *Xenopus* oocytes exhibits a variety of exchange modes including Cl^--HCO_3^-, Cl^--Ox^{2-}, SO_4^{2-}-Ox^{2-} which are DIDS sensitive and appear to be electrogenic [24, 33, 40, 42].

Because of the strong and unequivocal affinity for the oxalate anion and its disposition in the apical membranes of enterocytes, it would not be surprising to find that PAT-1 plays an important role in the transcellular transport of oxalate by mediating the initial uptake of oxalate from the lumen during absorption or by mediating oxalate efflux from the cell during secretion. The latter possibility is intriguing in light of the studies that demonstrate cAMP-dependent oxalate secretion by colonic epithelia [6, 7]. While a channel-like mechanism was proposed to mediate oxalate efflux across the apical membrane [21], this does not exclude the possibility of additional cAMP-dependent pathways like PAT-1 mediating oxalate efflux across the apical membrane.

Other members of the SLC26 gene family that have been reported to encode for proteins that accept oxalate as a substrate [26, 40] include Sat-1, sulfate anion transporter (SLC26A1) which is abundantly expressed in liver and in proximal tubule basolateral membranes [46, 47]. While interest in Sat-1 has focused on renal epithelia, where it serves as a SO_4^{2-}-anion exchanger with a high oxalate affinity [47], Sat-1 mRNA has been reported in caecal tissues of mice [48] and in human small intestine and colon [49]. Furthermore, we have recently found that Sat-1 mRNA is expressed in both the distal and proximal segments of the small and large intestine of healthy rats [50] and in post-confluent monolayers of Caco-2 cells [38] using real time RT-PCR. It is tempting to hypothesize that the Sat-1 protein, generated by mRNA we have detected, is targeted to the basolateral membrane of enterocytes and may thus represent the SO_4^{2}-Ox^{2-} (HCO_3^-) carrier detected using rabbit ileal basolateral membrane vesicles [19]. This

proposal is particularly attractive in that it provides a defined molecular anion exchange mechanism for oxalate transport across the basolateral membrane in intestinal epithelial cells, which naturally complements the roles of apical exchangers like PAT-1 (and perhaps others?) noted above.

The potential and relative importance of other members of the SCL26 family of anion exchangers to transepithelial oxalate transport in the intestine is not certain at the present time either because they have not been reported in intestinal segments and/or because their oxalate affinity is low or not defined. Thus, the SLC26A2 gene encoding the the diastrophic dysplasia sulfate transporter (DTDST) protein is relatively abundant in the human colon but less so in small intestine [51] and is present in Caco-2 monolayers [38] and rat intestine [50]; yet the affinity of this SO_4^{2-}-anion exchanger for oxalate has not been clearly established. The SLC26A4 gene encodes a Cl^--formate exchanger called Pendrin, which is highly expressed in renal proximal tubule apical membranes, and we have noted mRNA expression of this gene in Caco-2 cells [38], but Pendrin protein expressed in *Xenopus* oocytes does not transport oxalate or sulfate [52] anions. Likewise, there are several SLC26A genes that encode exchangers that are reported to accept oxalate as a substrate (SLC26A7–SLC26A11), however, expression of these genes in intestinal epithelia is not apparent at this time [26].

The SLC4 gene family also has members that encode for anion exchanger proteins that are found in intestinal epithelia [53], but are not currently regarded as playing a primary role in oxalate exchange in the intestine. Among these are the anion exchangers AE1, AE2, and AE3 (SLC4A1–SLC4A3), with AE1 being the prototypical stilbene-sensitive, Cl^--$HCO3^-$ exchanger most abundantly expressed in red blood cell plasma membranes—the venerable ''band3'' protein. In the rat, AE1 is also expressed in the apical membranes of ileum [54] and surface cells (not crypt) of the distal colon [30], while it was not detected in biopsy samples of human intestine [55]. The AE2 and AE3 proteins are expressed to varying degrees along the length of the gastrointestinal tract and generally on the basolateral aspect of the enterocytes in humans [55], rats [56] and mice [57], where they are involved with the regulation of intracellular pH. There are two reasons for mentioning these SLC4 family members in this discussion of intestinal oxalate transport. First, band3 or band3-like proteins were of interest in early transepithelial oxalate transport studies simply because they exhibited a DIDS-sensitivity reminiscent of oxalate transport in native epithelia [7, 17] and vesicles [18, 58], thereby giving an oxalate transporter a tangible molecular identity. Second, in red blood cells the band3 protein is clearly capable of exchanging chloride for oxalate with kinetics that suggest that Cl^- and oxalate compete for a common transport site with Cl^- affinity about three times that for oxalate [59]. Thus, while the AEs are generally characterized as monovalent anion exchangers, there are situations, especially with AE1, where they are involved in oxalate transport which may be relevant to intestinal oxalate across the apical membrane.

In closing this section on oxalate-anion exchangers two additional points should be noted that may affect our ultimate interpretation of the functional significance of these potential oxalate transport pathways. First, oxalate exchange with a counter ion can theoretically occur in either direction across a biological membrane depending on the magnitude and direction of the conjugate driving forces acting on the ions. Thus, whether a given exchanger can actually have a functional role in transcellular oxalate transport is determined by the electrochemical potentials of these ions across the membrane—a feature that has been largely ignored because it is relatively difficult to assess. Second, it is important to recognize that even though an exchanger does not exhibit the ability to transport the oxalate anion directly, it may still play an integral role in transepithelial oxalate fluxes. That is, the supporting roles played by other anion exchangers in maintaining gradients for ions that are counter transport partners with oxalate on ''oxalate exchangers'' is an issue that will likely continue to muddy experimental approaches and their interpretations, yet are crucial to a full understanding of oxalate transport at a given membrane beyond the molecular events of the exchange process.

Intestinal oxalate handling in animal models simulating disease states

Studies over the past decade have shown that alterations in oxalate movements across the intestine can occur in disease states. These alterations may involve a directional change in cellular oxalate transport as well as changes in both transcellular and paracellular pathways resulting in either enhanced oxalate absorption or secretion. This section will summarize the results of studies which have examined intestinal handling of oxalate, primarily in animal models simulating disease states. While the validity of these models, and models in general, can be debated, most notably absent is an authentic model for the rare genetic disease primary hyperoxaluria (PH). At best, only inferences can be drawn from the studies of the animal models exhibiting hyperoxaluria with hyperoxalemia, either with or without renal failure, which are some of the clinical manifestations of PH types 1 and 2. Studies focusing specifically on renal handling of oxalate following oral oxalate loading [2] will not be reviewed in this section in any depth.

Chronic renal failure

In the early 1990s, two studies using 5/6 nephrectomized rats provided evidence indicating that enteric excretion of oxalate occurs in chronic renal failure (CRF) [5, 60].

Normally, the primary route for excretion of oxalate is the healthy kidney but a small amount (~6%) of oxalate is excreted by enteric elimination [60]. In the first study, which followed the fate of ^{14}C-oxalate infused by a subcutaneous mini-osmotic pump, it was demonstrated that faecal excretion of the tracer was fivefold higher in CRF rats compared to controls [60]. In the second study [5], using a similar rat model, flux measurements of oxalate across isolated short-circuited intestinal tissues showed that basal oxalate absorption observed in the distal colon of control rats was reversed to net oxalate secretion in CRF. In contrast to the distal colonic segment, the direction of oxalate transport was not altered in other parts of the intestine in CRF [5]. While no significant changes in paracellular conductances were evident in the latter study, passive movement of oxalate from the blood into the gut lumen through this shunt is likely to occur in vivo along the entire intestine as plasma oxalate concentrations increase and a transepithelial oxalate gradient favors this directional movement. Subsequent studies on the 5/6 nephrectomized rat model showed that CRF-induced colonic oxalate secretion was segment specific and mediated by angiotensin II type I (AT_1) receptors [20] which were upregulated almost twofold in CRF colonic mucosa [61, 62]. Basal oxalate absorption could be restored by injecting CRF rats with the AT_1 receptor antagonist, losartan and it was also possible to simulate CRF-induced secretion of oxalate by acute serosal application of angiotensin II to in vitro tissue preparations [20]. A later study which focused on another rat model, in which CRF was induced by chronic hyperoxaluria, also confirmed adaptive colonic secretion/excretion of oxalate mediated by AT_1 receptors [63]. An important conclusion from this work was that hyperoxaluria, per se, does not induce alterations in colonic oxalate handling, but that hyperoxalemia together with some degree of renal insufficiency seems to be required to induce ANG II-mediated colonic secretion. Thus, it appears that oxalate elimination is balanced between the renal and enteric routes, and we suggest that by maximizing enteric elimination of oxalate, the burden of oxalate excretion via the kidneys will be reduced and consequently the risk of hyperoxaluria, oxalosis, and kidney failure will be attenuated.

Oxalate-associated diseases

There are several animal models available that simulate some of the clinical characteristics of the oxalate-associated diseases, namely stone disease and PH, and a number of these have been examined with respect to the intestinal handling of oxalate [64, 65]. In one study, rats were given oxalate challenges in a variety of ways (dietary supplementation, acute injection, ethylene glycol administration) to produce hyperoxaluria either with or without hyperoxalemia and without (ostensibly) altering renal function [64]. The results of these studies again demonstrated that hyperoxaluria, per se, does not induce changes in oxalate transport in either the proximal or distal colon. Rats fed oxalate in the diet (0.5% oxalate with 0.01% calcium) had hyperoxaluria comparable to the other groups but these rats, unlike the other groups, were not hyperoxalemic and colonic oxalate transport was normal. In the other groups, which had in common an elevation in plasma oxalate, colonic oxalate secretion was evident. Of significance is that these studies provided evidence for additional oxalate secretory pathways, independent of ANG II regulation and distinct from those electrogenic chloride secretory pathways previously implicated in oxalate secretion [64]. Whereas some degree of renal insufficiency seems to be required to induce ANG II-mediated colonic secretion, an elevation in plasma oxalate alone leads to colonic oxalate secretion that is largely independent of ANG II mediation.

Enteric hyperoxaluria

Since the malabsorption of bile salts and fatty acids is a common finding in enteric hyperoxaluria, early studies focused on the effects of these agents on mucosal permeability. Using various techniques, including perfused colonic segments from humans [66] and both perfused loops and everted gut sac preparations from rats [13, 67, 68], it was determined that dihydroxy bile salts and long chain hydroxy fatty acids increased oxalate absorption across the large intestine. Other in vitro experiments using isolated short-circuited colonic preparations from both rat [69] and rabbit [70, 71] demonstrated that there was an increase in bidirectional oxalate fluxes following bile salt or fatty acid addition. For example, the addition of 1–4 mM of taurochenodeoxycholate to the isolated rabbit distal colon resulted in enhanced paracellular passive flux of oxalate, while simultaneously inducing a transcellular net secretory flux [70, 71]. Similar effects were seen with even lower concentrations (0.25–2 mM) of the hydroxy fatty acid ricinoleate [70]. The relative contribution of the transcellular, active secretory flux of oxalate is likely to negligible in vivo, however, and absorption of oxalate by way of gradient-driven, passive diffusion between cells (paracellular) will predominate in proportion to the luminal activity of the oxalate ion. In other studies, in which oxalate solubility was determined in the presence of calcium and fatty acids likely to be malabsorbed [13, 72], the affinity of calcium for fatty acid was greater than for oxalate, which would lead to increased oxalate activity and absorption. Recently, a rat model involving a 50% small bowel resection coupled with a high oxalate diet (0.57% oxalate with 0.02% calcium) has been presented as a model of enteric hyperoxaluria [65]. While those rats with resections were hyperoxaluric compared to controls and both mucosal to serosal and net oxalate fluxes were increased in ileum, proximal, and distal colon, it is not clear whether the rats exhibited malabsorption of bile salts and/or steatorrhea in order to satisfy the strict definition of enteric hyperoxaluria (as opposed to simply enhanced dietary

absorption of oxalate). Thus, enteric hyperoxaluria is explained by a large increase in the passive, paracellular flux of oxalate from the gut lumen into the blood (induced by both bile salts and fatty acids) that is driven by intraluminal concentrations of oxalate which are also increased by fatty acid chelation with intraluminal calcium.

Factors influencing intestinal oxalate absorption

The questions of how much of dietary oxalate is absorbed along the alimentary tract and the factors that can influence this have been addressed in various ways by numerous investigators. In oxalate loading studies in humans, intestinal oxalate absorption has been shown to range between ~3% and ~55% of the load administered [14, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84]. In addition, several studies have shown that intestinal oxalate absorption is increased in stone formers [75, 82, 84, 85, 86, 87, 88] as well as in patients with ileal dysfunction and/or steatorrhea [76, 77, 78, 83, 84, 89, 90]. This wide range of published values likely reflects not only differences among individuals but also differences in the experimental approaches used in each study. Clearly, there are several factors that can influence the amount of oxalate absorbed in any one individual including the way the standardized loading test is administered. Oxalate loads have been administered with food or when fasting, as a simple oxalate solution or complexed in food, and in some studies, either $^{14}C_2$-oxalate [77, 84, 86, 87, 90, 91, 92] or $^{13}C_2$-oxalate [80, 81, 82] have also been used as tracers for measuring intestinal oxalate absorption. Certainly, there are advantages and disadvantages associated with every experimental approach. For example, in studies in which a meal is administered as an oxalate load, precise determination of the variable oxalate content of food can be difficult to determine due to the extraction procedures and analytical techniques used for oxalate quantitation of specific food items. It is also important to note that the mere quantitation of food oxalate does not provide any measure of the bioavailability of the oxalate in that foodstuff in vivo. Typically, however, in all studies, intestinal absorption is calculated from the increase in urinary oxalate excretion, following the oral load, expressed as a percentage of the load. Although oxalate absorption is a process that can occur along the length of the alimentary tract, several studies have shown that the peak urinary response occurs by $\sim 4 \pm 2$ h following the oral oxalate load [77, 92, 93, 94, 95, 96]. This is consistent with the notion that the bulk of dietary oxalate is absorbed in the upper part of the intestine in healthy individuals. In addition, patients with a total colectomy appear to excrete the same amount of urinary oxalate as individuals with an intact colon [96].

Calcium

Among the many intraluminal factors influencing intestinal oxalate absorption, the consensus is that intraluminal calcium has a significant effect because it can chelate oxalate within the gut lumen and thereby reduce oxalate absorption. Direct evidence for the effects of calcium on oxalate absorption has been derived from both human [79, 97, 98, 99, 100, 101, 102] and animal experiments [103, 104, 105, 106]. Essentially, all of these studies demonstrate that intestinal oxalate absorption is reduced when the intraluminal concentration of calcium is increased. Similarly, lowering intraluminal calcium results in enhanced intestinal absorption and renal excretion of oxalate [79, 80, 102, 107, 108, 109]. This inverse association between intraluminal calcium and oxalate has also been confirmed indirectly by numerous studies assessing dietary calcium intake and stone risk (hyperoxaluria) or occurrence of stones [110, 111, 112, 113, 114, 115]. Invariably, these studies have shown that dietary calcium intake is associated with a decreased risk of kidney stones. Interestingly, in a large prospective study of 91,731 women (34–59 years], Curhan et al. reported that supplemental calcium, rather than dietary calcium intake may increase stone risk and, by way of explanation, these investigators suggested that this different effect may be associated with the timing of calcium ingestion relative to the amount of oxalate consumed [111]. However, in both an earlier study [110] of 45,619 men (40–75 years) and in a more recent study [115] of 96,245 younger women (27–44 years), Curhan et al. report that supplemental calcium is not associated with risk of stone formation. Thus, in clinical practice today, compared to a decade ago, it is not considered prudent to impose severe dietary restrictions on dietary calcium when treating calcium oxalate stone disease. In contrast, calcium supplementation is effective in treating enteric hyperoxaluria for the reasons explained above [68, 72, 116, 117, 118, 119, 120].

Other dietary factors

Although the effects of calcium on intestinal oxalate absorption have received by far the most attention, there may be other dietary factors to consider including magnesium, vitamin B_6, ascorbate, and fibre, to name just a few. The associations between these less studied dietary factors and intestinal oxalate absorption are derived mainly from studies examining urinary oxalate excretion in stone forming and non-stone forming individuals combined with dietary assessment. To our knowledge, studies which directly measure intestinal oxalate transport in the presence or absence of these compounds have not been conducted.

Magnesium

Magnesium can bind both intraluminal and urinary oxalate and forms a more soluble salt than calcium oxalate; but similar to calcium, dietary magnesium has been reported to lower urinary oxalate excretion by reducing oxalate absorption [80, 93, 121]. In several

studies, urinary magnesium excretion was shown to be lower in stone formers compared to controls [122, 123, 124, 125, 126], but other studies reported no differences [122, 127, 128] and there is conflicting information regarding dietary magnesium intake in stone forming patients and whether it is lower than in non-stone forming controls [110, 111, 127]. Nonetheless, magnesium supplementation of magnesium-deficient patients forming stones appeared to reduce the incidence of stone recurrence [129, 130, 131]. It is not clear, however, whether this is due to an effect on intestinal oxalate absorption, on urinary oxalate chemistry, or a combination of both possibilities. A limited number of studies, using magnesium-deficient rats, have also yielded conflicting results regarding the effects of magnesium on intestinal oxalate absorption. In one study, it was concluded that magnesium-deficiency did not promote intestinal oxalate absorption [132] while another study demonstrated a significant increase in oxalate uptake across brush border membrane vesicles (BBMV) prepared from the intestines of magnesium-deficient rats [133]. Presently, the role of intraluminal magnesium and its impact on oxalate absorption appears less clear than its effects on the urinary chemistry of oxalate.

Pyridoxine

The role of pyridoxine as a cofactor for the enzymatic transamination of glyoxylate to glycine by alanine:glyoxylate aminotransferase (AGT) is well recognized because PH type 1 is caused by a deficiency of AGT [134]. Whether pyridoxine, or pyridoxine-deficiency, has any effects on intestinal absorption of oxalate is questionable. Intestinal oxalate uptake studies by Sharma et al. revealed increased bioavailabilty of oxalate from the gut of pyridoxine-deficient rats [135] which would lead to enhanced intestinal oxalate absorption. In another study, which examined oxalate uptake by BBMV, it was concluded that acute, sub-clinical, and chronic pyridoxine deficiency did not modify oxalate influx into BBMV [136]. The results from these studies are not inconsistent but clearly more studies are required to determine whether pyridoxine directly influences intestinal oxalate handling.

Ascorbic acid

The contribution of dietary ascorbic acid to urinary oxalate excretion is still controversial. Information from several large epidemiological studies found no association between ascorbate intake and stone risk [137, 138, 139, 140], which is in contrast to several investigators reporting a higher intake of ascorbate in stone formers compared to non-stone forming individuals [85, 127, 141, 142]. Many of the earlier experimental studies indicated that ascorbate supplements increased urinary oxalate excretion [143, 144, 145, 146, 147] and some, but not all, of these studies were criticized because of possible in vitro conversion of ascorbate to oxalate in the urine during and after it was collected. Yet other studies did not report elevations in oxalate excretion following ascorbate supplementation [148, 149, 150, 151] so the question remained. More recently, concern has been raised again based upon a number of new studies confirming that ascorbate ingestion can induce hyperoxaluria to varying degrees depending upon the dosage [152, 153, 154, 155, 156, 157]. Thus, the current consensus appears to be that stone forming patients should be cautioned to limit or avoid ascorbate supplements altogether.

Fibre

An inverse relationship appears to exist between dietary fibre and stone formation since stone formers have been reported to have significantly lower intakes of fibre [127, 158, 159]. Several studies have reported the positive effects of dietary fibre in reducing the intestinal absorption and urinary excretion of calcium in hypercalciuric patients [160, 161, 162, 163, 164, 165, 166], however, the role of dietary fibre in oxalate absorption and urinary oxalate excretion is not certain. In some of the studies that determined urinary oxalate excretion, either no changes [165, 166, 168], a reduction [167], or a slight increase [160] was observed in patients receiving supplemental dietary fibre. In a rat study, Ohkawa et al. [162] reported that urinary calcium excretion and its intestinal absorption were reduced significantly only when the rats were fed a high calcium diet. When rats were placed on a low calcium diet, this hypocalciuric effect did not occur and no information was presented on oxalate handling. Theoretically, if the mechanism of the hypocalciuric effect is due to intraluminal binding of calcium, an enhanced intestinal absorption and urinary excretion of oxalate would be expected to ensue. Perhaps, as Robertson [169] argued, the fibre network could trap calcium in the form of calcium oxalate crystals and thereby attenuate the bioavailability and absorption of oxalate. Suffice it to say, a direct effect of dietary fibre on intestinal oxalate handling has yet to be demonstrated.

Does *Oxalobacter* sp. play a role in intestinal handling of oxalate?

A number of intestinal microorganisms have been reported to degrade oxalate including *Eubacterium lentum* [170], *Enterococcus faecalis* , [171], lactic acid bacteria *Lactobacillus* spp., *Streptococcus thermophilus*, *Bifidobacterium infantis* [172], and *Oxalobacter formigenes* [173]. Because *O. formigenes* has received the most attention by investigators, this section will only focus on the role of this bacterium in intestinal handling of oxalate. For a review of the oxalate degrading bacteria, see Allison et al. [174].

Human studies

It was in 1980 that an anaerobic bacterium, with a substrate-specificity for oxalate, was isolated from rumen contents [175]. Later, when it was isolated from human feces and other animals, a new genus and species *O. formigenes*, was established to include these microorganisms [173]. In the intervening years compelling evidence emerged from a number of human and animal studies suggesting that *O. formigenes* can play an important role in reducing intestinal oxalate absorption. In most of the studies involving human subjects, the approach has been to determine whether the lack of *Oxalobacter* colonization can be associated with increased urinary oxalate excretion and stone formation. In one of the first reports (appearing in 1989), Doane et al. showed that urinary and faecal excretion of oxalate were significantly lower in a small group of women colonized with *Oxalobacter* compared to those who were not colonized [176], but curiously the difference in urinary oxalate excretion between the two groups disappeared when the study subjects were challenged with an oxalate load. As an explanation for these oxalate-loading results, we suggest that it is most likely a reduction in urinary oxalate would have occurred if these study subjects were acclimated to increasing dietary oxalate loads (see Animal studies below). In another study of patients with cystic fibrosis (CF), Sidhu et al. observed that seven of 43 CF patients were colonized with *Oxalobacter* and all seven had normal urinary oxalate excretion, while over half of the CF patients who were not colonized were found to be hyperoxaluric [177]. Several other studies have also shown that stone forming patients who are *Oxalobacter* -negative have significantly higher urinary oxalate excretion compared to *Oxalobacter* -positive patients [178, 179, 180, 181]. Further support for the role of *Oxalobacter* in protecting against stone disease was provided by another study which showed a direct correlation between the number of recurrent kidney stone episodes and the lack of *Oxalobacter* colonization [182]. This interesting correlation was later confirmed by two other studies of oxalate stone formers in north India [183, 184]. Yet, another approach used to determine the importance of *Oxalobacter* has been the measurement of oxalate degradation in faeces. Very low, or negligible rates of oxalate degradation were measured in faecal samples from a number of patient populations including stone formers [185], patients with inflammatory bowel disease [186], Crohn's disease [187], and patients with jejunoileal bypass [188]. These observations led Allison to suggest that the bile salt malabsorption and steatorrhea, which is common in these conditions, may result in the inhibition of colonic *Oxalobacter* activity [174]. This notion is certainly supported by the results of in vitro studies demonstrating that *Oxalobacter* is highly sensitive to low concentrations of the bile salt, deoxycholate [189, 190]. However, additional studies addressing the viability of *Oxalobacter* in vivo and the colonization status of patients with enteric hyperoxaluria are especially important because the colon is the location for both enhanced oxalate absorption and *Oxalobacter* colonization. Based upon the results of all of these studies, in our opinion, it is reasonable to consider the lack of intestinal *Oxalobacter* activity as a risk factor for hyperoxaluria and stone disease, but clearly the absence of this colonic bacteria alone is unlikely to be a direct cause of stone disease simply because of the multifactorial nature of the disease.

Currently, the ultimate question regarding the importance of *Oxalobacter* in intestinal handling in health and disease is whether this bacterium has application as a probiotic. In 2002, it was shown for the first time that a single oral dose of *O. formigenes* resulted in a reduction in urinary oxalate following an oxalate load in four human subjects and in two of these individuals, who were *Oxalobacter* -negative prior to the loading study, colonization was evident many months later [190]. Whether *Oxalobacter* or supplemental supplies of *Oxalobacter*, or its enzyme products, can therapeutically reduce urinary oxalate excretion and stone disease consistently in various patient populations needs to be proven in long-term studies.

Animal studies

Studies in animals and animal models have also provided useful information about the physiological relationship between *Oxalobacter* and oxalate metabolism. *Oxalobacter* has been isolated from ruminants and various other animals including wild rats, but it is generally not found in laboratory rats (see Allison et al. for a review [174]). In studies using cattle, and sheep [191, 192, 193, 194, 195, 196] and other non-ruminant herbivores including horses [197], swine, rabbits [198], and guinea pigs [189] it was demonstrated that exposure of animals to increasing amounts of dietary oxalate resulted in increased rates of oxalate degradation, suggesting that oxalate tolerance can be developed because of robust *Oxalobacter* activity leading to reduced intestinal oxalate absorption. While initial studies using laboratory rats failed to show that artificially colonized rats excreted lower amounts of urinary oxalate compared to controls [199], later studies showed more promise. Sidhu et al. were able to show that urinary oxalate excretion in hyperoxaluric rats (induced by dietary supplementation of oxalate) was significantly reduced by orally administering either encapsulated oxalate-degrading enzymes from *Oxalobacter* [182] or by administering viable whole *Oxalobacter* cells [200] for 2 weeks. In the latter study, increasing dosages of the bacterium were correlated with larger reductions in urinary oxalate excretion suggesting that probiotic treatment may be a potentially effective treatment for hyperoxaluria.

Pertinent questions regarding the factors that influence *Oxalobacter* colonization and how colonization is initiated have been addressed in a few studies using rats. In humans, apparently, infants less than 9 months old

are not yet colonized with *Oxalobacter* but almost all children become colonized by 6–8 years of age and 70% of adults are colonized [201]. Daniel et al. found that the rat's diet had to contain at least 3% oxalate in order to sustain *Oxalobacter* colonization [202] and Sidhu et al. observed that when oxalate is removed from the diet, artificially colonized rats lose colonization within 5 days [200]. Apart from the fact that it appears that a dietary source of oxalate is required to maintain colonization status, other factors such as intraluminal calcium and antibiotic treatment will also influence colonization status and *Oxalobacter* activity [174, 190]. The question of how colonization is initiated was addressed in a very recent report by Cornelius and Peck [203]. In a study using rats, these investigators concluded that intestinal colonization with *Oxalobacter* does not occur vertically from biological mother to pup; rather, transmission of the bacterium is horizontal via the environment. Residual questions prompted by these results are how this anaerobe survives an aerobic environment if transmission is via the faecal-oral route and how long can it survive in the environment.

The results of all of these animal studies are consistent with the human studies and with the notion that *Oxalobacter* can degrade intraluminal, dietary-derived oxalate and reduce the amount of oxalate available for absorption. However, a more provocative question that we have begun to address is whether *Oxalobacter* can derive oxalate from systemic sources, possibly by initiating or enhancing intestinal oxalate secretion. We have hypothesized that there is a physiological interaction between the bacterium and the gut transporting mucosa and the question is whether this microorganism has a strategic ability to optimize substrate availability within the intestinal lumen by locally modulating epithelial oxalate secretion. Preliminary evidence from our laboratory [50] suggests that this is the case. Urinary oxalate was reduced about 50% in hyperoxaluric rats (induced by ethylene glycol treatment) treated with encapsulated *Oxalobacter* enzymes compared to placebo treated rats. More importantly, the transport studies conducted using the distal colon from these rats exhibited net oxalate secretion in contrast to a net absorptive flux of oxalate in the placebo-treated group. Similar results were obtained in another pilot study when naturally colonized rats, with normal urinary oxalate excretion, were compared to rats not colonized [50]. These results are significant because they show: (1) support for the notion that *Oxalobacter* can derive oxalate from systemic sources by initiating colonic oxalate secretion, and (2) that the balance between renal and enteric excretion of endogenously-derived oxalate, can be manipulated.

Summary and conclusions

The body of knowledge regarding intestinal handling of oxalate and factors affecting oxalate absorption has grown substantially during the past decade or so. Whereas in the early 1990s, the ''thinking'' was that the intestine functioned purely in an oxalate absorptive mode, we now know that mechanisms for intestinal oxalate secretion exist and the intestine can play a significant role in adaptively excreting oxalate under certain conditions. The role of *Oxalobacter* sp. in oxalate metabolism, its physiological interactions with the intestinal mucosa, and its potential as a probiotic are rapidly being established and hold promise. In addition, the clinical management of oxalate stone disease is currently being guided by important observations from both epidemiological and experimental studies, most notably in the consideration of dietary calcium and ascorbate intake.

In the next few years, it is anticipated that the major advances in our understanding of intestinal oxalate handling will come from the molecular identification of transport mechanisms for oxalate along the intestine combined with functional studies so that we can understand the vectorial nature of oxalate transport in living cells and tissues. In addition, aided by newer molecular approaches, the regulatory mechanisms involved in the coordination of vectorial oxalate transport in either direction, will begin to be unraveled. It is anticipated that there will be newer approaches to effectively reduce oxalate absorption, or enhance intraluminal oxalate degradation, which would represent an advance in the clinical treatment of the oxalate associated diseases. Given the uncertainty of successful outcomes in any arena, however, what is certain is that the obdurate anion will continue to demand the curiosity and attention of many obstinate investigators for years to come.

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