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Oxalate transport and calcium oxalate renal stone disease

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Abstract Hyperoxaluria is considered to play a crucial role in calcium oxalate (CaOx) renal stone disease. The amount of oxalate excreted into the urine depends on intestinal absorption, endogenous production, renal clearance and renal tubular transport. Since a primary disorder has not been found so far in most CaOx stone formers and since oxalate is freely filtered at the glomerulus, most studies are presently focussed on alterations in epithelial oxalate transport pathways. Oxalate can be transported across an epithelium by the paracellular (passive) and transcellular (active) pathway. Oxalate transport across cellular membranes is mediated by anion-exchange transport proteins. A defect in the structure of these transport proteins could explain augmented transcellular oxalate transport. Little is known about the physiological regulation of oxalate transport. In this review cellular transport systems for oxalate will be summarized with special attention for the progress that has been made to study oxalate transport in a model of cultured renal tubule cells. Better understanding of the physiological processes that are involved in oxalate transport could yield information on the basis of which it might be possible to design new approaches for an effective treatment of CaOx stone disease.

Key words Idiopathic calcium oxalate nephrolithiasis · Transcellular oxalate transport · Kidney · Intestine · LLC-PK$_1$

Introduction

In urine that is supersaturated with respect to calcium oxalate (CaOx), the formation of crystals depends on the level and activity of urinary promoters and inhibitors of crystallization [33, 42, 51, 52, 57, 58, 74]. Renal stones are the ultimate result of crystals that have been retained in the kidney on basis of their dimensions or by their association with the renal tubular epithelium, allowing them to increase in size further [33, 42, 67]. Urinary oxalate is a much more important determinant of the level of supersaturation than calcium. Mild hyperoxaluria is a common finding in recurrent idiopathic CaOx stone formers [2, 9, 34, 41, 42, 52, 57–59, 66, 68, 69, 74, 75]. In male recurrent idiopathic stone formers it has been demonstrated that the amount and size of CaOx crystals and the severity of the disease is highly related to their urinary excretion of oxalate [51, 52].

The amount of oxalate that is excreted in the urine depends on dietary intake, endogenous production, intestinal absorption and renal transport [37, 41, 42, 68, 74, 75]. The endogenous production of oxalate, predominantly derived from the metabolism of glyoxylate and ascorbate, contributes importantly to the amount of oxalate that is excreted in the urine [41, 42, 59, 68, 74, 75]. However, in most CaOx stone formers a primary disorder in the metabolism of oxalate has not been demonstrated [42, 59, 68]. After the administration of oxalate-containing food, normally only a small amount of oxalate is absorbed from the gastrointestinal tract [59]. It has therefore been assumed that hyperoxaluria could result from an abnormally increased absorption of oxalate from the intestine [20, 22, 37, 42, 59, 68]. For this reason, stone formers are usually advised to avoid the consumption of oxalate-rich food. On the other hand, a number of clinical studies demonstrated that increased dietary intake did not lead to hyperoxaluria [3, 16, 50]. Secretion of oxalate by the renal tubule cells contributes to the overall urinary excretion of oxalate [10, 15, 31, 32, 47, 54, 73]. An abnormally enhanced secretory pathway for oxalate in the kidney could potentially lead to hyperoxaluria [9, 37, 59, 68].

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Much of the information about the mechanisms that are involved in cellular oxalate transport has been derived from studies with isolated membrane vesicles. However, transport processes in vesicles do not imperatively reflect transport in intact and functional epithelia [46]. Microperfused renal tubules have been used as a model for such an intact epithelium, but this approach is technically demanding and is limited by the small volume of the luminal compartment. Alternatively, transport studies can be performed under controlled conditions across sheets of polarized and functional epithelia mounted in a two-compartment system. A model to study oxalate transport across stripped intestinal tissue has already been presented [19–22]. Unfortunately, this approach is not feasible with renal tissue. Cultured epithelial cells could serve as an alternative in vitro model to study oxalate transport. Recent progress made in the development of a cell culture model of renal proximal tubule cells for the purpose of studying oxalate transport will be reviewed and discussed.

Methods to study oxalate transport

A variety of methods have been used to study the transport of oxalate, either in vivo or in vitro. In most of the earlier reports clearance studies and micropuncture experiments were employed, focussing on the determination of the fractional delivery of oxalate and the location of its secretion in the nephron [10, 15, 31, 32, 47, 54, 73].

Microperfusion is a highly sophisticated technique that has been used for in situ measurements of oxalate transport. In this method the tubular lumen and/or the peritubular blood capillaries are perfused with a solution containing radioactively labeled test compounds (e.g., oxalate) and inulin as a reference substance. Measurements of the disappearance of the test compounds relative to that of inulin yield valuable information about the kinetic parameters of the transport systems. Such studies may also be performed with isolated tubules [62].

Another approach is to mount excised tissue fragments containing a continuous epithelial cell layer into a chamber especially designed for studying transport processes. A major advantage of the use of such so-called Ussing chambers is that transepithelial fluxes can be performed under electrophysiologically controlled conditions using voltage clamping. Net transport is defined as the difference between the two unidirectional fluxes. This technique has been applied successfully to study oxalate transport across strips of intestinal tissue [19–22], but for practical reasons is difficult to perform with renal tissue.

Further dissection of the transport processes can be achieved by the use of isolated cellular membranes. The transcellular transport of solutes across intact polarized epithelia is complex and involves the movement of ions across the apical as well as the basolateral plasma membrane. Vesiculated membranes can be isolated from kidney homogenates by differential centrifugation and used for short-term [^{14}C]oxalate influx and efflux studies. Such studies have yielded specific information about the contribution of brush border and basolateral membranes to overall transepithelial oxalate transport [1, 27–30, 36, 38, 59, 77].

In the last few years, renal cell lines in culture have been increasingly used to study oxalate transport processes. Oxalate uptake was reported for the proximal tubule cell line LLC-PK$_1$ grown in monolayers in plastic culture dishes [69]. The disadvantage of this approach is that uptake can only be observed at the apical membrane. A major improvement was the introduction of culture methods in which cells can be grown on permeable porous supports. In general, epithelial cells cultured on porous supports in a two-compartment system tend to form highly polarized and differentiated monolayers that, compared to cells grown on conventional plastic substrates, mimic much better the morphological and functional characteristics of the epithelium found in vivo. This has also been observed for the renal cell lines LLC-PK$_1$ and MDCK, which both form polarized monolayers of differentiated cells when cultured on permeable supports (Figs. 1, 2). Such a model is, therefore, a superior system to study transport processes [17, 35, 55]. In addition, it allows the assessment of directional transepithelial solute fluxes from the apical to the basal compartment and vice versa. Solute secretion or absorption is indicated by the direction of net transport, which is defined as the difference between the unidirectional fluxes [17, 34, 35, 45, 48, 56, 66]. Since functional epithelia, especially those from the renal proximal tubule, are relatively leaky, the contribution of the paracellular pathway to overall transport of a small molecule is usually monitored by the simultaneous unidirectional transepithelial movement of an extracellular marker such as mannitol or inulin [45, 48]. Another approach is to study transepithelial ion transport under short-circuit conditions in Ussing chambers in which net transport of a charged substrate reflects transcellular rather than paracellular transport [12, 19–22]. In addition, this model system allows the separate assessment of initial influx rates of tracers at either the apical or basolateral plasma membrane.

Renal handling of oxalate

Oxalate is a small dicarboxylate ion ($C_2O_4^{2-}$) that is freely filtered at the glomerulus. As early as 1962 clearance studies in dogs by Cattel et al. showed that the amount of oxalate excreted into the urine was greater than the quantity that was filtered at the glomerulus,

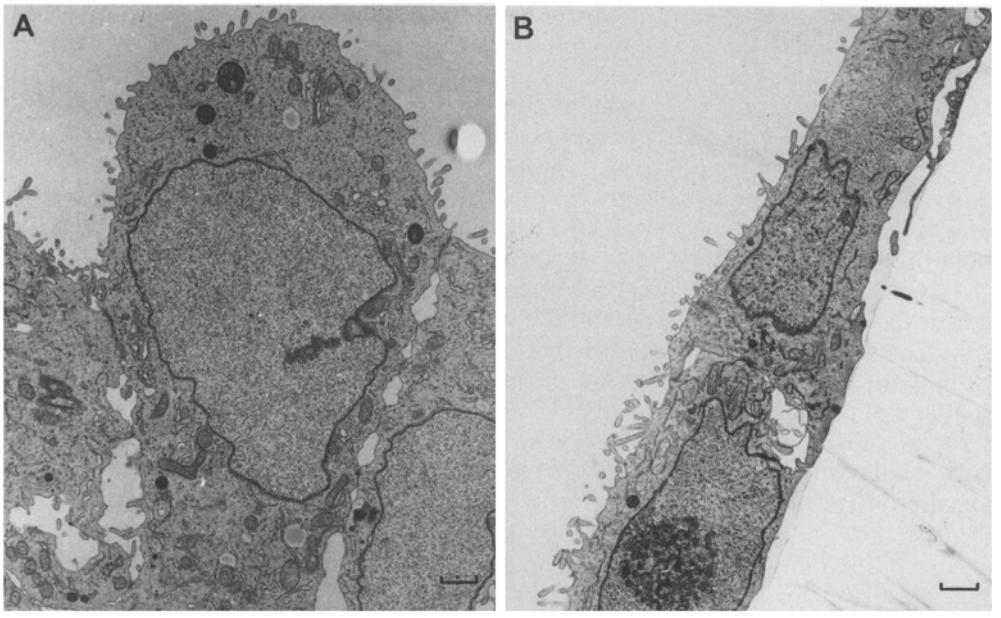

Fig. 1A, B Transmission electron microscopy images of MDCK (**A**) and LLC-PK_1 (**B**) cells cultured on a porous support in a two-compartment culture system

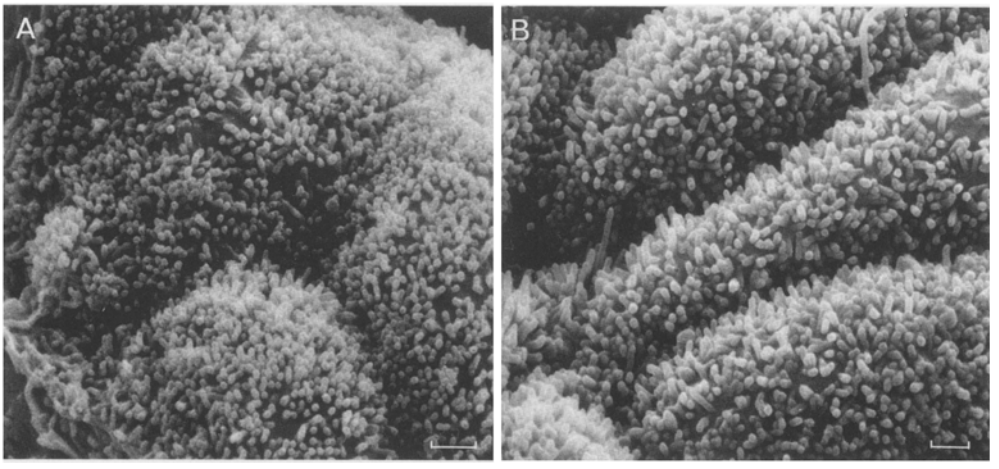

Fig. 2A, B Scanning electron microscopy images of MDCK (**A**) and LLC-PK_1 (**B**) cells cultured on a porous support in a two-compartment culture system

indicating active tubular secretion of oxalate [10]. In addition, stopped-flow analysis indicated that the tubular secretion of oxalate is localized at the proximal tubule, whereas there was no evidence for active reabsorption of oxalate [10]. In the late 1970s a number of investigators studied the renal handling of oxalate in rats by clearance, micropuncture and microperfusion techniques [15, 31, 32, 54, 73]. Greger et al. [15] suggested that bidirectional movement of oxalate takes place in the rat proximal convoluted tubule (PCT) and net oxalate secretion in the straight segment of the proximal nephron. Weinman et al. [73], however, proposed that oxalate is secreted in early portions of the rat PCT and undergoes bidirectional transport in later segments. Later the same group suggested the existence of more than one oxalate secretory system in early and late segments of the rat PCT [31, 32]. Clearance and microperfusion experiments in rabbits confirmed that oxalate undergoes net renal secretion in the proximal tubule and showed that this secretion is more extensive in early and late segments than in the straight segment of the PCT. Moreover, these authors found a significant internephronal heterogeneity for oxalate secretion reflected by the observation that superficial nephrons showed a greater rate of secretion than juxtamedullary nephrons [54]. Based on the results from microperfusion experiments, Tremaine et al. [61] suggested that the transport system for oxalate in the chicken kidney is separate from those handling *p*-aminohippurate (PAH) and uric acid. Tubular secretion of oxalate has also been reported in man [47]. Taken together, these studies indicate that oxalate is actively secreted in the mammalian renal proximal tubule.

Transcellular movements of substrates from the peritubular capillaries to the tubular fluid require their crossing of two barriers: the basolateral plasma

membrane, which is in contact with the internal milieu facing the blood supply, and the apical plasma membrane, which is in contact with the external milieu and faces the tubular fluid. The transport of oxalate across these membranes is mediated by anion-exchange mechanisms. The plasma membranes of most epithelial cells contain anion-exchange systems that are structurally and functionally related to the prototype of plasma membrane anion exchange, erythrocyte band 3 protein [1]. Ullrich et al. used tubular and/or capillary microperfusion techniques in the rat kidney to differentiate subtypes of organic anion transporters in the proximal tubule and described the following transport systems: the lactate transport system, the sulfate transport system, the dicarboxylate transport system and the PAH transport system [14, 62, 63]. On the basis of its structure and negative charge distance (3–4 Å), oxalate was found to be a substrate for the sulfate transport system at the basolateral plasma membrane [11, 62–64]. At the apical membrane, electroneutral secretion of oxalate was proposed to occur in exchange for Na^+-dependent reabsorption of sulfate [11].

Studies performed with isolated membrane vesicles demonstrated the presence of distinct oxalate transporters in the mammalian proximal tubule. In rat renal cortex basolateral membrane vesicles, Löw et al. [38] found a sulfate/bicarbonate anion exchanger that also has affinity for oxalate. This observation was confirmed later by Kuo et al. [36], who evaluated the pathway for oxalate in rabbit basolateral cortex membrane vesicles and demonstrated oxalate exchange for bicarbonate and sulfate. In rabbit renal cortex apical membrane vesicles, Karniski and Aronson [28] reported chloride/formate exchange as well as chloride/oxalate and formate/oxalate exchange. In rat renal proximal brush border vesicles the presence of an oxalate/OH^- exchanger was demonstrated [77].

Intestinal handling of oxalate

Since oxalate handling in the gastrointestinal tract can influence the amount of oxalate reaching the kidney, it is relevant to consider intestinal oxalate transport as a factor that potentially contributes to the nephrolithiasis process. Hatch and coworkers have described a series of experiments in which oxalate transport was studied in tissue strips by the Ussing chamber technique [19, 20, 22]. Using short-circuited conditions it was shown that in rabbit intestinal tissues a net secretory (serosal to mucosal) flux occurred in jejunum, distal ileum and proximal colon, whereas a net absorptive (mucosal to serosal) flux was observed in the distal colon [22]. Intestinal oxalate transport appeared to involve anion-exchange mechanisms and to be dependent on the movement of other electrolytes. Studies performed with isolated membrane vesicles have provided evidence that anion exchangers are present both in the brush border (oxalate/OH^- and oxalate (formate)/chloride [29]) and in the basolateral membrane (oxalate (sulfate)/bicarbonate [29]) of the rabbit ileum. Furthermore, oxalate transport was responsive to various external stimuli. For instance, exposure of distal colon to dibutyryl-cAMP resulted in a secretory rather than an absorptive flux, probably involving enhanced oxalate uptake at the serosal membrane via the Na^+–K^+–$2Cl^-$ cotransport system [22]. Recently, also the stomach has been suggested as an additional site for oxalate absorption in the gastrointestinal tract [23]. It is unclear, however, whether the passive nonionic diffusion of oxalic acid under the experimental conditions used is representative of the conditions found in vivo, and therefore this observation should be confirmed by further investigations.

Role of oxalate in NaCl transport

The proximal convoluted tubule (PCT) is responsible for the reabsorption of most of the filtered NaCl [39]. In microperfusion studies in the rat chloride transport in the early and late PCT was found to be 300–600 and 200–300 peq/mm/min, respectively [76]. A major portion of chloride reabsorption in the PCT is attributable to active transcellular transport [5, 6, 49, 76]. Electroneutral, transcellular reabsorption of NaCl in the PCT has been suggested to be affected by a mechanism coupling Na^+/H^+ exchange to chloride/$base^-$ exchange, in which $base^-$ represents formate or oxalate [6, 53]. Karniski and Aronson proposed [27] that chloride/formate exchange with recycling of formic acid is a potential mechanism for active chloride absorption across the apical membrane in epithelia and later a similar mechanism was found for chloride/oxalate exchange [1, 40, 70, 71] (Fig. 3). The physiological role of oxalate in NaCl and fluid reabsorption in the rat PCT was recently confirmed in microperfusion experiments performed by Wareing and Green [72]. These authors found that addition of oxalate to the luminal perfusate in concentrations below 50 μM stimulated fluid reabsorption in a concentration-dependent manner. Their data suggested that the mechanism involving chloride/oxalate exchange was distinct from the mechanism which uses chloride/formate exchange. Furthermore, these authors raised the possibility that although plasma oxalate concentrations (±2–5 μM) are low compared to ultrafiltrate chloride concentrations (±120–140 mM), alterations in oxalate (or formate) plasma concentrations may have a highly significant effect on NaCl absorption. It is possible that the filtered portion of oxalate participates in the effective absorption of NaCl by recirculating at the luminal membrane via a mechanism that does not necessarily contribute to oxalate secretion. It is also possible, however, that a secretory portion of oxalate that is transported from the peritubular capillaries to the apical membrane of

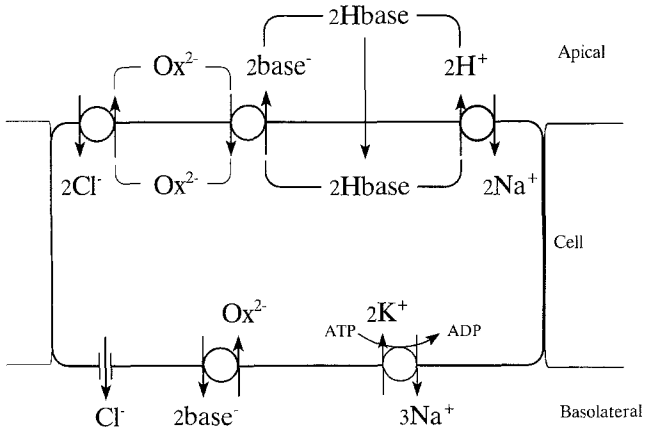

Fig. 3 Putative mechanisms explaining the involvement of oxalate in mediated and coupled electroneutral NaCl reabsorption in the renal proximal nephron. A sodium gradient induced by the Na^+/K^+-ATPase pump at the basolateral membrane stimulates sodium entry at the apical membrane mediated by the Na^+/H^+ antiporter. Chloride enters the cell at the apical membrane in exchange for oxalate (or formate) and exits from the cell at the basolateral membrane by mechanisms that are not completely identified, but possibly by chloride channels. Oxalate enters the cell at the basolateral membrane in exchange for a base (HCO_3^- or SO_4^{2-}). To absorb NaCl effectively, oxalate recirculates at the apical membrane in exchange for a base (HCO_3^- or OH^-) or SO_4^{2-} that is cotransported with sodium (not included in the figure). By this mechanism micromolar amounts of oxalate can contribute to the reabsorption of millimolar amounts of NaCl

the renal proximal tubules contributes to this process. In the latter case, it is conceivable that a transcellular transport pathway exists that couples oxalate secretion to chloride and fluid reabsorption (Fig. 3). Alternatively, the transport of oxalate in the kidney could also take place by mechanisms that are independent of chloride absorption. Oxalate secretion could be mediated by the cellular entry of oxalate in exchange for sulfate and/or bicarbonate at the basolateral membrane, followed by the cellular exit of oxalate in exchange for sulfate, bicarbonate or hydroxyl ions at the apical membrane. Detailed studies are required to further elucidate these electrolyte transport mechanisms.

Oxalate transport in cell culture

Cultured epithelial cell lines derived from renal tubules are convenient in vitro models to study the expression and modulation of transport systems. In a homogeneous dynamic population of cells transport studies can be performed under strictly controlled experimental conditions. These studies can confirm and extend the results that are obtained from membrane vesicles and microperfusion studies. Improved knowledge of the mechanisms that are involved in renal oxalate transport could shed some light on the processes that lead to hyperoxaluria. A number of renal cell lines with differentiated characteristics of distinct nephronal segment are presently available [18]. Most transport systems that have been described in renal proximal tubule cell lines are sodium dependent such as Na^+ cotransport of sugar, amino acid and phosphate [48]. Oxalate seems to participate in the renal handling of other physiologically important anions such as chloride, sulfate and bicarbonate. Anion transport in the kidney is heterogeneous with respect to nephronal segments and is highly regulated to obtain overall electrolyte homeostasis. These considerations indicate that, in order to study transport of oxalate in a cell culture model, basic knowledge of other electrolyte transport mechanisms in the kidney is required and experiments should be performed under well-controlled conditions.

Several attempts have been made to develop a cell culture model to study the classical organic anion transport system in the kidney. This transport system in the late PCT has affinity for a large number of chemically unrelated substances [62] and is an effective means for secreting endogenous or xenobiotic toxicants into the urine [7]. PAH is commonly used as a convenient probe for this system, which is therefore often referred to as the PAH transport system. The progress that has been made in studying PAH transport in renal cell lines is given as an example of anion transport studies in culture, though the mechanisms involved are not necessarily identical to those required for the transport of other anions such as oxalate. Vectorial transport of PAH has been demonstrated in a number of culture models as diverse as primary cultured flounder proximal tubular cells [12] and the established cell line OK-1, derived from the American opossum kidney [25]. In contrast, PAH transport was not observed in primary cultures of rabbit proximal tubule cells and in the proximal tubule cell line LLC-PK_1 [43]. The transcellular PAH transport across monolayers of the OK-1 cell line was found to be unidirectional in the basal to apical direction [25]. This is in agreement with the presence of a PAH/dicarboxylate exchange system in the basolateral membrane of OK-1 cells [60]. PAH transport by this system is dependent on high intracellular α-ketoglutarate concentrations that effectively *trans*-stimulate PAH uptake at the basolateral membrane. An α-ketoglutarate gradient is generated by coupled Na^+-dicarboxylate transport and by intracellular metabolism [62]. The PAH/dicarboxylate exchanger has no affinity for oxalate [60, 62]. Evidently, different transport proteins and driving forces are involved in renal transport of PAH and of oxalate.

Attempts have been made by our group [66] and by others [34, 69] to study oxalate transport in pig kidney LLC-PK_1 cells, a cell line that has been used extensively as a model for the renal proximal tubule [26]. We measured unidirectional fluxes of [^{14}C]oxalate and, in addition, the initial uptake rate of oxalate at both plasma membranes. These studies were based on experiments performed by Rabito et al. [48] and Mullin et al. [45], who demonstrated the presence of a number

of Na^+-dependent transport mechanisms in this cell line. The functional integrity and correct polarization of the epithelial layer was indicated by the demonstration of sugar uptake at the apical membrane. Nevertheless, under these experimental conditions, we were unable to detect a net flux of oxalate across these monolayers and there was no difference in initial rates of oxalate uptake at either plasma membrane. From these results we concluded that, under the conditions used, LLC-PK_1 cells did not exhibit a specific oxalate transport system. Since we could not exclude the possibility that oxalate anion fluxes were influenced by solute resistance induced, for example, by the natural apical negative potential difference (1 mV) in LLC-PK_1, these studies were recently repeated under short-circuit conditions (unpublished observation). The results, however, did not alter the conclusions that were derived from our initial studies on oxalate transport in this cell line.

Wandzilak et al. [69] studied oxalate transport characteristics in LLC-PK_1 cells cultured on a solid substrate and reported that oxalate uptake was inhibited in the presence of sulfate, chloride, bicarbonate or anion-exchange inhibitors. Koul et al. [34] performed short-term oxalate influx and efflux studies in LLC-PK_1 cells. Their results indicated the presence of an oxalate/chloride exchanger at the apical and an oxalate/sulfate (bicarbonate) exchanger at the basolateral surface. Although the results of these studies demonstrated that the basic elements for oxalate transport are present in the membranes of LLC-PK_1 cells, these experiments do not appreciably extend our knowledge beyond the level gained from isolated plasma membranes and do not demonstrate the occurrence of net oxalate secretion into the apical compartment. The advantage of an intact and functional epithelium in culture is that it allows the measurement of secretory or absorptive tracer fluxes across the epithelium. The absence of net oxalate transport in LLC-PK_1 cells in our studies suggested to us that in addition to anion-exchange mechanisms other factors are possibly required to allow net secretion of oxalate.

Whether or not the LLC-PK_1 cell line is a suitable model to study oxalate transport remains rather questionable. Transport processes that occur in the proximal tubule are predominantly determined by the composition of the tubular fluid [49]. It has been described that when a proximal tubule is perfused with a glomerular ultrafiltrate sodium-dependent transport processes predominate. When, on the other hand, a proximal tubule is perfused with a pure NaCl solution the reabsorption of sodium and chloride will prevail [5]. It might be of vital importance to know the exact location of oxalate transport within the proximal tubule since the composition of the tubular fluid is different in the various segments (S1, S2 and S3). Since LLC-PK_1 cells are apparently equipped to transport oxalate, it seems worthwhile to continue the efforts to reveal directed oxalate fluxes across these monolayers under experimental conditions that mimic the conditions found in vivo.

Confluent monolayers of LLC-PK_1 cells cultured on a solid substrate form cyst-like structures called "domes" which accumulate between the cells and the growth substrate [18]. It has been recognized that dome formation represents active reabsorption of NaCl and fluid. When LLC-PK_1 cells are cultured on a permeable support, dome formation is not observed since the fluid will be transported across the epithelium to the basal compartment (unpublished observation). It has been reported that monolayers of LLC-PK_1 cells cultured in a two-compartment culture system develop an apical negative potential difference that becomes apical positive within 12 h [44]. This voltage polarity shift correlated in time with a fall in apical glucose levels and with the development of an apical-to-basolateral-directed chloride gradient. These processes seem to mimic the renal PCT, where the preferential reabsorption followed by the apical depletion of glucose, amino acids, sulfate, bicarbonate and phosphate leads to a shift in voltage polarity (apical negative to apical positive) which favors the absorption of chloride and fluid [5, 6]. The initial step for transcellular oxalate transport is the exchange of oxalate for sulfate and/or bicarbonate at the basolateral membrane. This process is energetically driven by the Na^+/K^+-ATPase pump system, which provides the Na^+ gradient by which sulfate and bicarbonate are reabsorbed from the tubular fluid. Chloride/oxalate exchange is one of the proposed mechanisms for the cellular exit of oxalate at the apical membrane. It could therefore be speculated that secretion of oxalate can only be expected when the physiological conditions are also favorable for chloride absorption. Since chloride transport in the proximal tubule is known to be controlled by a number of hormones [13], it is conceivable that the transport of oxalate and chloride can be induced by the addition of specific hormones to our transport buffer system.

Intrarenal physical forces such as colloid osmotic and hydraulic pressures contribute to transport processes in the proximal tubule [49]. In microperfusion experiments it has been demonstrated that the peritubular protein concentration modulates active NaCl reabsorption [4] and that serum albumin facilitated tubular secretion of organic anions [8]. These observations suggest that the addition of serum or albumin to our buffer system during transport studies might be needed in order to better mimic the in vivo situation.

In conclusion, several experimental conditions still need to be examined in order to answer the question whether the LLC-PK_1 cell line is a valuable model for studying renal oxalate transport. In addition, it could be worthwhile evaluating the possible application of other renal proximal tubule cell lines or primary cultures for this purpose. Finally, the question might be

raised whether renal oxalate secretion will only take place in the proximal tubule. Under our standard conditions, MDCK cells, a cell line that has extensively been used as model for the renal distal/collecting duct, were indeed unable to transport oxalate [66]. It has been reported, however, that chloride secretion by MDCK cells via the Na-K-2Cl cotransport system can be induced by hormones [55]. Since this transport system, known to be present in the distal nephron [65], resembles the chloride transport system in the distal colon [24], which could be stimulated by cAMP to secrete oxalate [22], it is conceivable that appropriate hormonal stimulation might also induce oxalate transport across MDCK monolayers. Such a finding would challenge the dogma that oxalate is secreted exclusively in the renal proximal tubule.

Possible disorders in cellular oxalate transport

Hyperoxaluria could result from (1) increased oxalate absorption in the intestine which, in turn, leads to the secretion of a larger amount of oxalate by the kidney. It could also be speculated that (2) oxalate secretion is primarily increased in the kidney which thereby facilitates oxalate uptake in the intestine. Another possibility is that (3) intestinal oxalate absorption and renal oxalate secretion are both constitutively increased. The exchange of oxalate across the membrane of red blood cells is measured as a possible marker for inheritable defective cellular anion transport mechanisms [2, 9]. Red blood cells are preloaded with oxalate, after which the exchange of "cold" oxalate for radioactively labeled oxalate is measured in time. Baggio et al. [2] and Borsatti [9] suggested that a defect in the function of the oxalate anion-exchanger found in red blood cells of recurrent stone formers could represent a generalized membrane abnormality that might also be present in the membranes of other oxalate-transporting cells. Ullrich and coworkers [14, 62, 63] demonstrated that, although anion-exchange mechanisms can be divided into distinct groups, these transport proteins are not very specific with respect to their substrates. It is therefore indeed conceivable that a minor, possibly inherited, difference in the structure of a band-3-related transport protein would alter the affinity of a transport system for oxalate in such a way that oxalate absorption in the intestine and/or secretion in the kidney is increased. Alternatively, Hatch et al. [19, 20, 22] suggested that dietary or absorptive hyperoxaluria could be the consequence of a decrease in the secretory component of intestinal oxalate transport. The relationship between intestinal absorption and renal excretion of oxalate in the regulation of the mass balance of oxalate was convincingly demonstrated by these authors. In rats with experimentally induced chronic renal failure, oxalate was secreted by the distal colon while in untreated rats oxalate was absorbed in this segment. It should be noticed, however, that these experiments were performed with rodent tissue and do not necessarily reflect the situation in humans.

Concluding remarks

For a long time a physiological role for oxalate was unknown and oxalate was considered to be an apparently useless end product of metabolism. From the recent observations that oxalate might be actively involved in and coupled to renal NaCl reabsorption, it is tempting to speculate that alterations in the renal handling of NaCl may have consequences for the amount of oxalate that is secreted into the urine. The bulk of chloride reabsorption in the kidney takes place in the early and late renal proximal tubule [76], locations also proposed for oxalate secretion. In order to maintain electrolyte homeostasis, the concerted handling of oxalate in the kidney and intestine could compensate for relatively small disorders in the overall electrolyte balance and hyperoxaluria could be the result of such combined actions.

The reduction of the total amount of oxalate that is excreted into the urine of recurrent stone formers could be the key to prevention or successful treatment of CaOx urolithiasis. Knowledge of the mechanisms that are involved in the renal transport of oxalate is essential in the development of such new therapies. A model of cultured renal tubular cells that is suitable for studying oxalate transport could be a valuable tool for obtaining such detailed information.

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