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LLC-PK$_1$ cells as a model system to study proximal tubule transport of water and other compounds relevant for renal stone disease

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Abstract LLC-PK$_1$ cells were cultured on a permeable support in a two-compartment culture system. Confluent monolayers received an ultrafiltrate-like solution at the apical side and a plasma-like solution at the basolateral side. The distribution of various solutes, including phosphate, calcium, and oxalate over both compartments was measured in time. The transport of water was monitored by alterations in fluid concentrations of radiolabeled inulin. Bicarbonate, glucose, and phosphate were transported rapidly from the apical to basolateral side of the monolayer. Sodium and chloride were reabsorbed without major consequences for the osmolality in the apical and basal fluid. Calcium and potassium were also reabsorbed, but to a smaller extent than sodium. The luminal concentration of oxalate gradually increased to values that were at least three times higher (12.0 ± 0.4 μmol/l) than those in the contraluminal fluid (3.8 ± 0.1 μmol/l). However, since the luminal rise of oxalate completely matched the rise of inulin in the apical fluid this appeared to be the passive consequence of active water reabsorption rather than of net directed oxalate transport. The LLC-PK$_1$ model could prove useful to study the regulation of proximal tubule water transport and its effect on luminal stone salt concentrations under different physiological conditions.

Key words LLC-PK$_1$ · Water transport · Oxalate Stone salts · Nephrolithiasis

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Introduction

Calcium oxalate and phosphate salts are extremely poorly soluble in biological fluids. In renal tubular fluid their concentration products easily exceed the value needed for spontaneous crystal formation. Although these crystals by themselves are not necessarily harmful because they are usually eliminated with the urine, their possible retention could ultimately lead to the formation of a renal stone [11, 12, 13, 17, 29, 30, 31]. Causes for retention may be formation of crystals inside the nephron at an abnormal site, in abnormal numbers or with abnormal sizes and aberrant interactions between crystals and the epithelial cells lining the renal tubules [11–14, 29].

The first event in stone development is probably the precipitation of calcium phosphate crystals in the limb of Henle. This is followed by calcium oxalate crystallization in the distal tubule and collecting ducts, whereby previously formed calcium phosphate crystals may act as a heterogenous nucleator [11, 12, 16]. The first crystallization step is sensitive to variations in the concentration of calcium and phosphate at the end of the proximal tubule, the latter is sensitive to calcium and oxalate concentrations and pH in the distal tubule and collecting ducts [12, 13, 16, 27, 28]. Most filtered phosphate is actively reabsorbed in the proximal tubule. Factors that influence phosphate reabsorption include the amount of filtered phosphate, dietary intake, the level of ionized calcium and acid-base status [25]. Also the bulk of filtered calcium is reabsorbed in the proximal tubule. Calcium transport is primarily passive in this segment of the nephron and is influenced by the amount of filtered calcium, dietary cal-

cium and phosphate intake, calcium complexation in the tubular fluid, defects in sodium and water reabsorption, and acid-base status [25]. No recent data are available for site-specific oxalate handling in the kidney. This information is important given the impact that, due to the high calcium to oxalate ratio in the tubular fluid, relatively small changes in oxalate concentrations have on the level of urinary calcium oxalate supersaturation [20].

Taken together, proximal tubular handling of water, calcium, phosphate, and oxalate is an important variable determining the risk of crystal formation in the nephron. The question is whether abnormalities in this handling can explain the formation of too many and too large particles as witnessed in recurrent stone former urines [17]. Such abnormal proximal tubule functions may not be apparent in the final collected urine, since processes occuring in late nephron parts will counteract them to guard overall homeostasis [25]. On a 24-h basis, the values for calcium, phosphate, and oxalate excretion in idiopathic stone formers do not differ much from those in non-stone formers. Mean urinary oxalate values, for example, may be slightly increased but the ranges largely overlap [10, 20, 22, 26]. However, the combination of high normal excretions of these stone salts does give significantly increased levels of tubule fluid supersaturation [4, 27]. In addition, transient differences in solute concentrations which may result from proximal tubule dysfunction have been reported. After an oxalate load, oxalate is excreted rapidly in the urine and with high peaks [18], but the peaks are higher in stone formers [8] and also urinary phosphate levels are higher in postprandial urine of stone-forming individuals [23, 24]. In view of these data, transport functions of the proximal tubule are of interest for idiopathic calcium stone formation.

In the present study, we have investigated proximal tubule transport in LLC-PK_1 cells, a cell line that has retained in culture many of the characteristics of the mammalian proximal tubule [9, 19] Although this cell line has been used extensively to study transport processes, as far as we know, our approach to study the reabsorption of water has not been reported previously, particularly in relation to its effect on luminal stone salt concentrations. The alterations in the composition of the luminal fluid are interpreted in the light of crystallization risks in the nephron.

Materials and methods

LLC-PK_1 cells were seeded at a high density (1×10^6 cells/4.52 cm^2) on permeable supports in a two-compartment culture system (Transwell-clear polyester membrane, Costar Badhoevedorp, The Netherlands). Transport experiments were performed 8–9 days post-seeding. Earlier we demonstrated that this length of time is needed for the development of functional LLC-PK_1 monolayers [32]. Fresh culture medium, Dulbecco's modified MEM (DMEM, Gibco BRL, Paisley, UK) supplemented with 10% fetal calf serum (FCS) was added to the cells every other day. To study solute transport, DMEM supplemented with 10 mM HEPES, pH 7.4, but without serum, representative for ultrafiltrate, was applied to the apical compartment, whereas DMEM, 10 mM HEPES, pH 7.4, supplemented with 10% FCS, resembling the peritubular fluid, was applied to the basal compartment. The osmolality of both solutions was approximately 335 mosmol/kg. The initial volumes in the apical and basal compartment were 1500 and 2600 µl, respectively. These volumes were chosen such that artificial fluid movement, caused by hydraulic pressure differences and unrelated to cellular processes, was prevented as much as possible. Fluid from both compartments was collected after 24 and 48 h for the determination of the various solutes. In another series of experiments 0.1 µCi/ml [^{14}C]oxalate (final concentration 5 µM) or 0.1 µCi/ml [^{14}C]-labeled α-methylglucoside (αMG, a non-metabolizable glucose-analog) in a final concentration of 1 mM were applied to both compartments and their distribution measured in time (radioactively labeled compounds obtained from Amersham International, Buckinghamshire, UK). In addition, the transepithelial transport of water (J_v) was monitored using radiolabeled inulin ([^{3}H]inulin). Since the monolayers are practically impermeable for relatively large molecules such as inulin, alterations in [^{3}H]inulin concentrations are indicative for the transepithelial movement of water. The apical and basal compartment both received 5 µCi/ml [^{3}H]inulin. To determine fluid transport (J_v), a 100-µl aliquot was taken from the apical and basal fluid at different time points and counted in a ß-scintillation counter. The volume (V_t) in µl is calculated as follows: $V_t = dpm_0 \cdot V_0/dpm_t$, in which $dpm_t = dpm/100\mu l$ at t, V_0 is the initial volume in µl, and dpm_0 is the amount of radioactivity per 100µl at the start of the experiment. Fluid transport (J_v) in $\mu l/h \cdot cm^2$ is than calculated by: $J_v = (V_0 - V_t) \cdot (t \cdot A)^{-1}$, in which t = time (h) and A = the area of the filter inserts (4.52 cm^2). It should be noticed that the relatively long incubation times (24 and 48 h) in this study were required to measure water transport-induced alterations in fluid [^{3}H]inulin concentrations. To avoid fluid evaporation as much as possible during this period, the studies were performed in a humidified atmosphere and care was taken that the incubator was opened only for sampling. The various solute concentrations and osmolality were measured by standard clinical chemical methods.

The measured solute concentrations can be interpreted in several ways. The change in apical concentration will have relevance to the risk for crystal formation and retention. Alterations in absolute amounts in a compartment, obtained by multiplying the concentration of a solute with the volume at a given time, are indicative for net reabsorption or secretion. Changes in the absolute amounts of the apical plus basal compartment indicate net production or metabolism. To compare our findings with in vivo data, the ratio of apical fluid concentration at a certain time point (AF_t) to initial apical fluid (AF_0) concentration is calculated (AF_t:AF_0) in analogy to the tubular fluid to ultrafiltrate concentration ratio (TF:UF) [25].

Results

Solute concentrations

The concentrations of sodium and chloride in both compartments did not vary much throughout the experiment (Table 1). The AF_t:AF_0 concentration ratios for sodium and chloride were slightly increased from 1.0 at t = 0 to 1.05 and 1.11, respectively after 48 h, which most likely is explained by some fluid evaporation (Fig. 1). The luminal concentrations of bicarbonate, glucose, and phosphate were already dramatically reduced after 24 h and further declined thereafter (Table 1). The basal fluid concentrations of bicarbonate and glucose also decrease in time, whereas that of phosphate rises (Table 1). The AF_t:AF_0 ratios of bicarbonate, glucose and phosphate at 48 h decreased to

Table 1 Time-dependent solute transport by LLC-PK_1 cells. The apical compartment received DMEM without serum, representative for ultrafiltrate and the basal compartment received DMEM containing 10% serum to resemble the renal peritubular space ($n = 6$)

t	Sodium (mM)		Chloride (mM)		Bicarbonate (mM)		Glucose (mM)		Phosphate (mM)		Potassium (mM)		Calcium (mM)		Osmolality (mosmol/l)		pH	
	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal
0 h	153.8 ± 1.3	156.6 ± 1.0	119.7 ± 0.7	119.4 ± 0.8	18.1 ± 0.3	17.7 ± 0.3	24.1 ± 0.2	23.1 ± 0.1	0.80 ± 0.06	0.83 ± 0.06	5.10 ± 0.04	5.13 ± 0.03	1.66 ± 0.01	1.65 ± 0.03	334 ± 3	336 ± 3	7.4 ± 0.1	7.4 ± 0.1
24 h	155.6 ± 0.8	163.8 ± 1.6	129.4 ± 1.1	125.6 ± 0.7	4.8 ± 0.5	12.0 ± 0.8	7.1 ± 2.0	21.0 ± 0.1	0.23 ± 0.06	0.83 ± 0.06	7.29 ± 0.03	4.77 ± 0.1	2.66 ± 0.1	1.46 ± 0.01	334 ± 4	336 ± 3	7.4 ± 0.1	7.3 ± 0.1
48 h	161.4 ± 3.4	170.9 ± 0.4	132.6 ± 2.61	132.8 ± 1.7	1.3 ± 0.4	8.1 ± 0.5	0.07 ± 0.06	17.1 ± 0.4	0.10 ± 0.0	1.13 ± 0.06	7.19 ± 0.6	5.28 ± 0.1	3.31 ± 0.03	1.57 ± 0.05	351 ± 1	349 ± 1	7.2 ± 0.1	7.2 ± 0.1

0.07, 0.003 and 0.13, respectively (Fig. 1). Luminal fluid potassium and calcium were elevated in time, whereas they remained practically unaltered in the basolateral fluid (Table 1). The AF_t:AF_0 concentration ratios for potassium and calcium at 48 h increased to 1.41 and 1.99, respectively (Fig. 1). The osmolality in both compartments remained practically unaltered during the course of the experiment (Table 1). The pH remained unaltered in both compartments during the first 24 h, whereas after 48 h the fluid in both compartments acidified slightly (Table 1). The total amount of apically applied [^{14}C]α-MG is almost quantitatively transported to the basolateral fluid over time (Table 2), reflected by a AF_t:AF_0 ratio of 0.07 after 48 h (Fig. 1). Luminal oxalate, on the other hand, showed a time-dependent increase which was accompanied by decreased values at the basolateral side of the monolayers (Table 2), resulting in AF_t:AF_0 concentration ratios of 1.7 and 2.4, after 24 and 48 h, respectively (Fig. 1).

Fig. 1 Alterations in the composition of the apical fluid can be expressed as concentration ratio between the apical fluid at the start (AF_0) and end (AF_t) of an incubation period (AF_0:AF_t). When these data are compared with the ultrafiltrate (UF) to tubular fluid (TF) concentration ratios (TF:UF) in the mammalian proximal tubule [25], there is a striking similarity between these in vivo data and the data obtained from our cell culture model. The reabsorption of essential nutrients is indicated by decreased AF_0:AF_t ratio of glucose, bicarbonate and phosphate. In spite of the relatively large amount of fluid that is transported from the apical to basal compartment, little change is observed in AF_0:AF_t ratio of sodium and chloride, indicating isosmotic reabsorption of NaCl. The AF_0:AF_t ratio for oxalate is similar to that for inulin, indicating that the concentration of oxalate increases in the apical compartment due to water reabsorption

Table 2 Handling of α-MG, oxalate and inulin by LLC-PK$_1$ cells. DMEM without serum was applied to the apical compartment, whereas the basal compartment received DMEM containing 10% serum. Both compartments received [^{14}C]oxalate or [^{14}C]α-MG and the distribution of tracers was measured in time. In double-label experiments the distribution of [^{3}H]inulin (dpm/100 µl) was monitored to measure the transepithelial movement of water (J_v) (n = 6)

t	α-MG (mM)		Oxalate (µM)		Inulin (dpm/100 µl)	
	Apical	Basal	Apical	Basal	Apical	Basal
0 h	1.0 ± 0.05	0.9 ± 0.03	5.0 ± 0.1	5.0 ± 0.1	965 ± 55	950 ± 40
24 h	0.02 ± 0.008	1.2 ± 0.04	8.7 ± 0.2	4.0 ± 0.2	1631 ± 96	741 ± 28
48 h	0.07 ± 0.03	1.2 ± 0.06	12.0 ± 0.4	3.8 ± 0.07	2325 ± 69	676 ± 30

Fluid transport (J_v)

As calculated from changes in [^{3}H]inulin concentrations (Table 2 and Fig. 2), the volume in the basal compartment steadily increased over time from 2600 µl at t = 0 to 2920 ± 120 µl at 8 h (not included), 3399 ± 130 µl at 24 h, and 3726 ± 163 µl at 48 h (Table 2), 24 and 48 h, whereas in the apical compartment the fluid concomittantly decreased from 1500 µl at t = 0, to 1201 ± 60, 939 ± 56 and 658 ± 191 µl at respectively t = 8 (not included in Table 2), 24 and 48 h. The apparent rise in total volume (apical plus basal compartment), which after 48 h approximates to 109% of the initial total volume (= 4100 µl), most likely is to be ascribed to some fluid evaporation that slightly increased the concentration of radiolabeled inulin, which is also reflected in a slight overall rise in osmolality (Table 1). When the reabsorption of water was monitored continuously it appeared that J_v was linear up to 8 h at a rate of 8.1 ± 1.0 µl cm^{-2} h^{-1} (not shown), to decrease to 5.2 ± 0.3 and 3.0 ± 0.1 µl cm^{-2} h^{-1}, after 24 and 48 h, respectively.

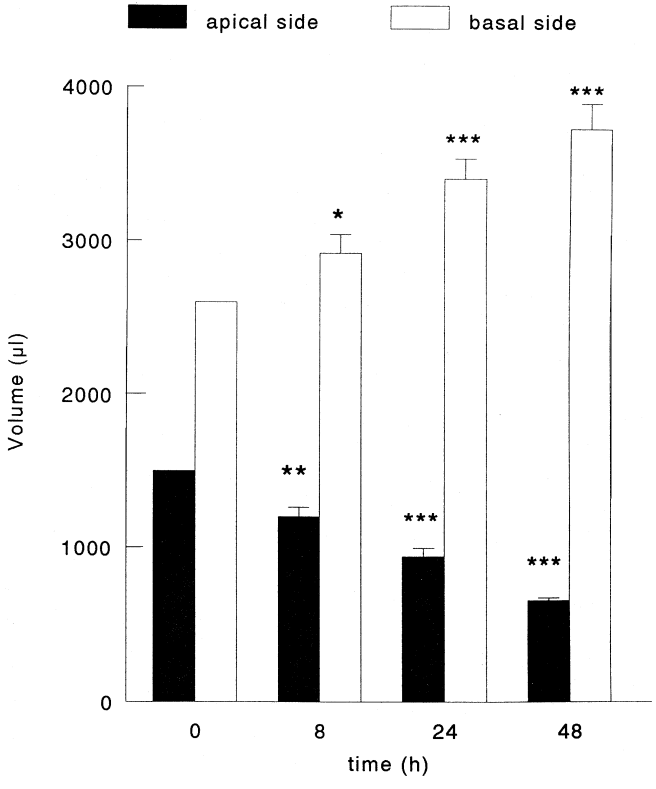

Fig. 2 Volume in the apical and basal compartment after 0, 8, 24 and 48 h, as calculated from changes in the [^{3}H]inulin concentrations. During this time period the cells received an ultrafiltrate-like solution at the apical side and the same solution including serum at the basolateral side of the monolayers. A decrease in the apical volume is observed and is accompanied by a volume increase in the opposite compartment, indicating that water is transported from the apical to the basal compartment. Results from a representative experiment, values are means ± SD of three independent measurements. *,**,***. Compared with the amount at 0, the fluid significantly decreased or increased in the apical and basolateral compartment, respectively, analyzed with Student's t-test (*0.025 > P > 0.01, **P < 0.01, ***P < 0.001)

Net solute transport

When these time-dependent volume changes are used to calculate absolute solute amounts in the compartments, it becomes evident that in spite of unaltered sodium and chloride concentrations, net NaCl transport occured from the apical to basal compartment. Such calculations also show that, for example, after 48 h, only 3% of the bicarbonate, 0.1% of the glucose, and 5.5% of the phosphate are still present in the apical fluid. In contrast to phosphate and α-MG, reabsorbed bicarbonate and glucose are not quantitatively recovered in the basal fluid. It also becomes clear that in spite of the rise in their luminal concentration, the absolute amount of potassium and calcium is reduced in the apical fluid. Although the concentration of oxalate in the apical compartment reached values more than threefold higher then those in the contraluminal compartment (Table 2), calculation of the absolute amounts at the different time points showed that this is not caused by net oxalate transport (7.5 ± 0.15, 7.72 ± 0.18, and 7.48 ± 0.25 nmol luminal oxalate at t = 0, 24 and 48 h, respectively).

Discussion

Proximal tubular functions may play a crucial role in calcium oxalate nephrolithiasis. The handling of phosphate, calcium, and water by the proximal tubule influences the risk that calcium phosphate crystallizes in the loop of Henle [12, 13]. The presence of calcium phosphate particles and the composition of the fluid

emerging from the loop of Henle in turn influence the risk of calcium oxalate crystallization in the distal tubule and collecting ducts [12, 28]. It is therefore of interest in urolithiasis research to study the composition of the fluid leaving the proximal tubules and how it is regulated. Here we assessed whether some of these transport functions can be studied in LLC-PK_1 cells. Although this cell line has been used widely during the past decade to study many transport mechanisms, these studies did not specifically address the possible relationship between processes involved in renal stone disease.

LLC-PK_1 cells were allowed to process an ultrafiltrate-like solution over time, during which we examined fluid transport (J_v), alterations in osmolality and pH, and the vectorial movement of solutes, including compounds that play an important role in stone formation such as calcium, phosphate, and oxalate. The various solutes were processed in a specific manner by the cells. Luminal fluid bicarbonate, glucose and phosphate rapidly decreased, whereas sodium and chloride remained practically unaltered and potassium and calcium increased. During the same time period, however, the luminal concentration of [^{3}H]inulin, an extracellular marker that diffuses little or not at all through the paracellular shunt pathway, increased more than twofold, whereas in the basal fluid it decreased, indicating that fluid was transported from the apical to basolateral side of the monolayers. Experiments in which bovine serum albumin (BSA, 6%) was added to either the apical or basal compartment, to both sites of the monolayer, or omitted from both compartments (data not shown), indicated that J_v was not influenced in this study by the presence or absence of serum proteins. The transport of water was recognized earlier in LLC-PK_1 cells by the formation of "domes" which are fluid-filled blisters caused by the transepithelial apical to basal directed transport of water that becomes trapped between the cultured cell layer and the solid culture dish [7]. In agreement with our data, analysis of the basolateral fluid collected from such domes by micropuncture showed that the concentrations of sodium and chloride were nearly the same in the dome fluid and the apical medium, while bicarbonate, glucose, and phosphate were actively reabsorbed. Our results indicate that NaCl and water are transported isosmotically from the apical to basal compartment, which is supported by the observation that the osmolality in both compartments remained practically unchanged during the complete course of the experiment (Table 1). Calcium and potassium are also reabsorbed but to a lesser extent than NaCl (Table 1), suggesting that their transport is passive and lags behind that of NaCl and water. At the end of the proximal tubule, the tubular fluid (TF):ultrafiltrate (UF) concentration ratios for calcium and potassium indeed are higher than that for sodium [25]. It should be emphasized, however, that under the experimental conditions used the various transport processes most likely are influenced by the alterations that take place over time. After 48 h, for example, glucose, bicarbonate, and phosphate are almost completely depleted from the apical compartment and, in addition, the fluid in both compartments was slightly acidified at this time-point. Since metabolic acidosis partitions potassium out of cells, this may have contributed to the lower reabsorption of potassium as compared with sodium. Whereas most reabsorbed solutes are transported almost quantitatively from the apical to basal compartment, glucose and bicarbonate are not completely recovered at the basolateral side of the monolayers. Since apically applied α-MG, which is a non-metabolizable analog of glucose, is almost quantitatively transported to the basal compartment; this indicates that an amount of glucose is lost by cellular metabolism. The reduction in bicarbonate most likely is explained by CO_2 release after H_2CO_3 formation and its subsequent dissociation in CO_2 and H_2O. It should be noticed that when we performed these experiments with MDCK cells, the luminal concentrations of bicarbonate, glucose, and phosphate were largely unaffected and nor was inulin significantly changed (data not shown), indicating that the above described results are specific for cells with proximal tubule characteristics.

Next, we studied the handling of oxalate by LLC-PK_1 cells. In previous studies, we demonstrated with more convential methods that oxalate is not preferentially transported to either side of LLC-PK_1 monolayers [32, 33]. Nevertheless, others found that LLC-PK_1 cells express oxalate transport proteins, including an apical membrane oxalate/chloride exchanger and basolateral membrane oxalate/sulphate and oxalate/bicarbonate exchangers [15]. In the present study, it was observed that the luminal concentration of radiolabeled oxalate gradually increased to values that after 48 h were at least threefold higher than those in the basal compartment (Table 2). Although this increase in apical oxalate suggested that oxalate was secreted, the rise in oxalate appeared to match that of inulin (Table 2), indicating that the luminal increase of oxalate is the passive consequence of water reabsorption. Thus, again we found that there is no net directed transport of oxalate across LLC-PK_1 monolayers.

What is the significance of these findings to stone formation? Since the phosphate concentration is a main determinant of the first nucleation of calcium phosphate in the loop of Henle, the regulation of phosphate reabsorption in the proximal tubule is of importance. It was shown recently that stone formers frequently have an inappropriately high postprandial, parathyrine hormone (PTH)-independent phosphaturia [23, 24]. The risk for calcium phosphate formation will be increased by diminished proximal tubule phosphate reabsorption. Indirectly, this will also speed up the formation of calcium oxalate crystals further on in the nephron [12, 13]. Small increases in the time a crystal spends inside a tubule can have magnifying effect on its size and subsequent risk for retention [12]. The site where crystals are formed initially may also be of importance. Several data point out that the epithelium in late nephron parts is protected against

particle adhesion [6, 29]. So, perhaps a shift in crystal formation to earlier parts of the nephron, that might not be protected from crystal binding, increases the risk for crystal retention.

Variations in oxalate concentration are especially important as they have a disproportional effect on the calcium oxalate formation product [20]. After filtration, oxalate might undergo bidirectional transport along the renal tubules [34]. For a long time, calculation of the renal clearance of oxalate was hampered by the lack of reliable methods to measure the relatively low concentrations of oxalate in plasma. Only recently this vital information in the understanding of the renal handling of oxalate is becomming available. Two studies, in which the clearance of oxalate was examined in relatively large populations of calcium stone formers and healthy individuals, showed that the mean oxalate/creatinine clearance ratio in both groups was close to unity [10, 26]. From these studies it was concluded that the mild hyperoxaluria observed in many patients may result from enhanced intestinal absorption or increased endogeneous production, but not from an abnormal renal handling of oxalate. Two other studies, however, in which smaller but more homogeneous groups of hyperoxaluric stone formers were investigated, found an oxalate/creatinine ratio above unity, suggesting net renal oxalate secretion [8, 22]. Taken together, it is still not clear whether or not there exists a transcellular renal transport pathway that contributes to the urinary excretion of oxalate. Perhaps net oxalate secretion only occurs in patients with a specific cellular transport defect [8] or under specific physiological conditions. For example, large increases in net acid production during metabolic acidosis or after a diet high in animal protein, causes compensatory mechanisms that contribute significantly to the risk of stone formation [1, 2]. A mild metabolic acidosis leads to decreased urinary citrate and increased urinary phosphate, calcium and, according to some reports, also oxalate [2]. The model system presented in this paper seems especially suitable to study the cellular handling of citrate and stone salts during acid-base disturbances. So far, our results demonstrate that under normal conditions there exists an equilibrium in the bidirectional transepithelial movement of oxalate across functional LLC-PK_1 monolayers. These results are in agreement with an oxalate/inulin (creatinine) ratio that is equal to unity (Fig. 1). Although the monolayers are relatively leaky, an apical to basal directed oxalate concentration gradient is generated, which is induced by water reabsorption. This observation indicates that oxalate becomes trapped at the luminal side of the monolayers.

The transport of water in the kidney has an important effect on filtered waste products. Intratubular concentrations of xenobiotics that are to be eliminated by the kidney gradually increase as a result of renal water reabsorption [5]. Although this common physiological process also has a large impact on the concentration of oxalate and thus on the formation product of its calcium salt, it has received little attention as a possible factor in the pathophysiology of renal stone disease. Approximately 99% of the water filtered at the glomerulus is reabsorbed by the kidney [25]. Even in the absence of active secretion, this process will lead to approximately a 100-fold increase in the tubule fluid oxalate concentration up to the collecting ducts. Assuming that the concentration of oxalate in ultrafiltrate is comparable to that in plasma (2–5 μM), this implies that along the length of the nephron the concentration of oxalate will rise passively to about 200–500 μM in the collecting duct [12]. The question is whether an essential physiological process such as renal water transport could be altered in stone-forming individuals. Evidence has emerged that apical membrane anion exchange mechanisms play an important role in the reabsorption of salt and water in the kidney [3]. The transport of NaCl in proximal and distal tubules of rodents is effected by a process that couples apical Na^+/H^+ exchange to chloride/oxalate (formate) exchange. Oxalate was found to stimulate NaCl and water reabsorption by recycling across the luminal membrane whereby anions required for chloride exchange are continually replenished intracellularly. Although speculative, increased ion exchange activity in the nephron could lead to enhanced water reabsorption resulting in higher luminal oxalate concentrations and the subsequent precipitation of stone salts in earlier segments of the nephron. Since the urine volume is fine-regulated in the collecting duct, higher levels of water reabsorption in early segments may be without consequences for urine volume and go unnoticed. In other words, it is possible that in stone-forming individuals the tubular fluid reaches the collecting duct more concentrated than in healthy subjects.

In summary, the results from this study demonstrate that functional monolayers of LLC-PK_1 cells process an ultrafiltrate-like solution, with stone salt concentration within the normal range, in a strikingly similar way to that described in the mammalian renal proximal tubule. Although there is no net directed transport of oxalate, its concentration in the luminal fluid increases as a passive consequence of isosmotic water reabsorption. This model may prove useful to study the regulation of NaCl and water transport in the proximal tubule, the effect of different calcium concentrations on the transport of phosphate, as well as the effect of metabolic acidosis on the cellular handling of compounds that are involved in renal stone disease. This information may provide new insights in processes that take place in the renal proximal tubule which may influence the risk of crystallization and stone formation in later segments of the nephron.

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