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Renal handling of citrate

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Intracellular citrate is a central component of the tricarboxylic acid cycle; its excretion in the urine was utilized by Krebs and coworkers to demonstrate this biochemical pathway in whole animals. Subsequently, urinary citrate has been viewed as a "window" on renal metabolism [1]. Additionally, renal physiologists and biochemists have been intrigued for decades by the dramatic changes in urinary citrate which occur with changes in acid-base homeostasis. In recent years, renal handling of citrate and citrate excretion in the urine have attracted renewed interest because of several considerations. First, modern techniques in renal physiology (such as, transport studies in brush border membrane vesicles and perfused proximal tubules) have provided insights into the cellular and molecular mechanisms of citrate transport [2]. Also, excretion of urinary citrate (and other organic anions) has been recognized to influence systemic acid-base status, at least in certain species [3]. And perhaps most importantly, urinary citrate has been increasingly recognized as an important endogenous inhibitor of calcium nephrolithiasis [4]. This review will focus on the renal handling of citrate, particularly the mechanisms of citrate transport in the proximal tubule, and briefly discuss other aspects of citrate metabolism.

Citrate is a tricarboxylic acid with pKa's of 2.9, 4.3, and 5.6.

$$\text{Citric Acid} \rightarrow \text{citrate}^{-1} \rightarrow \text{citrate}^{-2} \rightarrow \text{citrate}^{-3}$$

Since the highest pKa of citrate is significantly below physiologic pH, citrate is predominantly trivalent (citrate^{-3}) in plasma (Fig. 1). Plasma levels of total citrate are low, averaging 0.1 mM in normal subjects in a recent study [5]. Also, much of plasma (and urine) citrate is complexed to calcium, magnesium and sodium [6, 7]. The significance of this complexation for citrate transport has not been addressed. Plasma citrate appears to be relatively independent of normal dietary intake of citrate, probably because of rapid metabolism after gut absorption. However, plasma citrate does increase after oral citrate loads [8, 9]. Besides intestinal reabsorption of citrate, bone is the other major source (or reservoir) of plasma citrate. Utilization of citrate occurs chiefly in two organs, the liver and the kidney [10].

The importance of the kidneys in the metabolism of circulating citrate is illustrated by the fact that plasma citrate concentrations in experimental animals rise after nephrectomy (returning to normal after 24 hours) [11]. The citrate utilized by the kidneys is supplied predominantly by reabsorption of filtered citrate, with peritubular uptake of citrate accounting for the remainder (up to 30 to 40%) of citrate utilized by the kidneys [12]. Citrate is thought to be freely filterable at the glomerulus. In humans, 65 to 90% of the filtered citrate is reabsorbed; therefore 10 to 35% of filtered citrate is excreted in the urine. In most laboratory species, the fractional excretion of citrate is much less (such as, approximately 1% in the dog) [1, 5, 12]. Citrate reabsorption in animal studies in vivo does not appear to be saturated until plasma levels and filtered loads of citrate are raised several-fold [12]. If all of the citrate taken up by reabsorption of filtered citrate and by peritubular uptake of citrate were completely metabolized, citrate could provide approximately 10% of renal oxidative metabolism in humans [13]; higher estimates have been made for other species. Most of citrate taken up by the kidney does appear to be completely metabolized to CO_2 [12]. Therefore, citrate utilization represents a significant component of renal oxidative metabolism. Extensive studies of citrate metabolism in the kidney have been performed [reviewed in 1, 10, 14] but are beyond the scope of this review. Most of the oxidation of citrate probably takes place in the proximal tubule. The thick ascending limb, the only other nephron segment examined in detail, does not appear to metabolize citrate [15, 16]. Renal cell concentrations of citrate are higher than plasma levels of citrate, consistent with active uptake into renal cells [1, 17]. Urinary citrate is probably derived solely from filtered citrate which is not reabsorbed. Citrate secretion has not been demonstrated except during infusion of malate which may both interfere with citrate transport and alter intracellular metabolism [12, 18]. In most circumstances, urinary citrate is influenced relatively little by plasma citrate, compared to other factors discussed below [19].

Citrate transport in the kidney

Citrate reabsorption

Early stop-flow studies suggested that the major site of citrate reabsorption is the proximal tubule (Fig. 2) [20, 21]. However, one stop-flow study using adult baboons suggested significant citrate reabsorption in the distal nephron [22]. Recent studies of ours using isolated perfused rabbit nephron segments indicated that the proximal convoluted tubule and the proximal straight tubule reabsorbed citrate, whereas the thick ascending limb and cortical collecting tubule did not [18].

The mechanism of citrate reabsorption in the proximal tubule has been characterized in exquisite detail by studies utilizing rabbit brush border membrane vesicles [2]. The rate of citrate transport in the brush border membrane vesicles has been found to exceed that of sugars and amino acids studied under

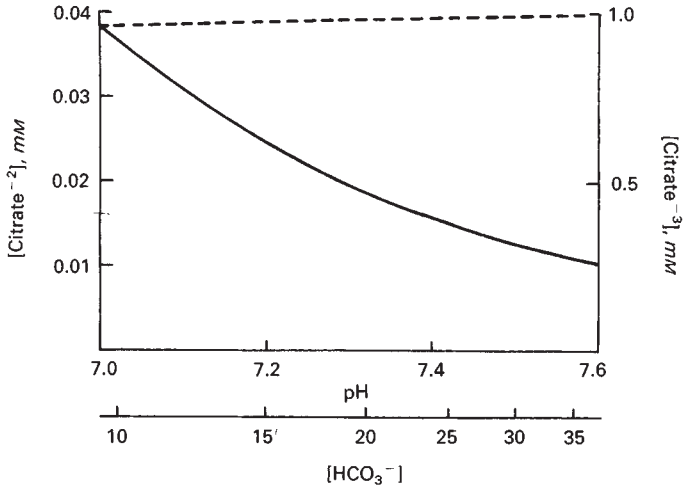

Fig. 1. *Calculated divalent and trivalent citrate concentrations, citrate^{-2} (—) and citrate^{-3} (---), respectively, as a function of pH and corresponding bicarbonate concentration with a pCO_2 equal 40 mm Hg.* Note that the scales for the two ionic species are nearly two orders of magnitude different with citrate^{-3} concentrations being much larger than citrate^{-2}. The assumed total citrate concentration is 1 mM and the pKa_3 of citrate is 5.6. Note that in a physiologic pH range citrate^{-3} changes very little with pH. However, citrate^{-2} concentrations change dramatically with pH in this range.

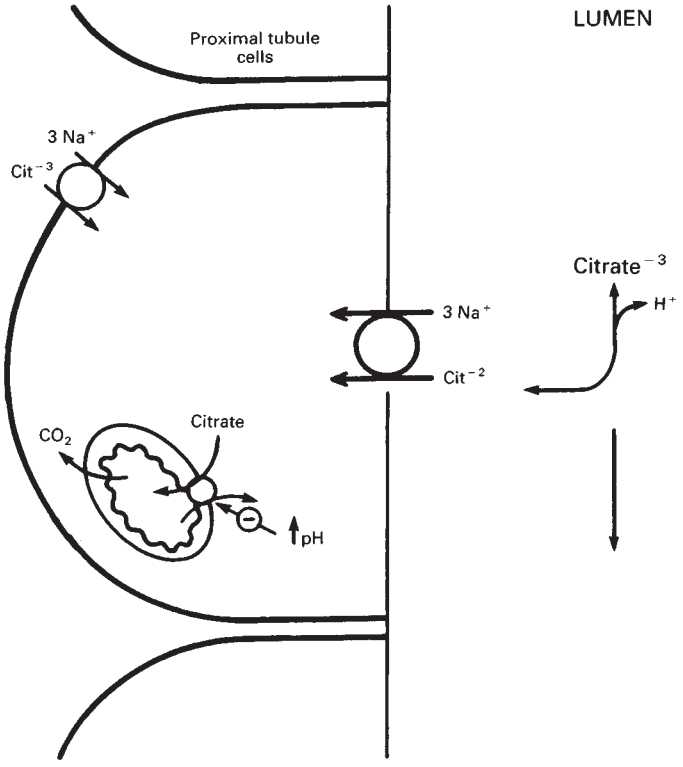

Fig. 2. *Model of proximal tubule cell handling of citrate.* Citrate is reabsorbed as the divalent species across the apical membrane but may be taken up across the peritubular membrane as the trivalent form. pH has effects not only on citrate entry into mitochondria but also has major effects on the concentration of the transported species. Note that the relative magnitude of citrate reabsorption across the apical membrane is greater than that of citrate uptake across the basolateral membrane.

similar conditions [23]. The apical membrane transporter responsible for citrate transport accepts a variety of Krebs cycle intermediates; in fact, the carrier has higher affinity for the dicarboxylate succinate [23, 24]. Most data, in fact, suggests that citrate is transported as the divalent species, citrate^{-2}. Citrate uptake is sodium dependent and electrogenic such that positive charge is transported. The stoichiometry is 3 Na^+ transported for each citrate^{-2} transported [reviewed in 2]. The more predominant trivalent species citrate^{-3} may, in fact, act as a competitive inhibitor of divalent citrate transport [25]. The affinity of the carrier for both citrate and the dicarboxylate succinate is quite high (K_m's of 0.21 and 0.05 mM, respectively) [24]. However, if divalent citrate is the only transported species as suggested, then the affinity is much high (K_m is much lower) than estimated from the total citrate concentration since the divalent species is present at a concentration less than 10% of the total citrate at physiologic pH. The predominant transport of divalent citrate^{-2} will produce a marked pH dependence of total citrate transport (as discussed in greater detail below) since the concentration of citrate^{-2} is markedly pH dependent in the physiologic pH range (Fig. 1). The specific molecular details of the kinetics, selectivity, and binding characteristics of the citrate or dicarboxylate carrier in the proximal tubule have been investigated [reviewed in 2]. Citrate does not appear to interact with a variety of other organic anion transport pathways in the apical membrane, including that for monocarboxylates.

Many of the important aspects of citrate transport have been confirmed by studies of intact proximal tubules. In our studies of rabbit proximal convoluted tubules perfused in vitro, approximately 80% of citrate transport was inhibited by ouabain, consistent with a dependency on sodium transport [18]. The component of citrate reabsorption which was not blocked by ouabain may represent a modest passive permeability to citrate. The magnitude of citrate reabsorption in the perfused proximal tubule exceeds the expected load of filtered citrate by several-fold. However, in contrast to the brush border membrane vesicle studies cited above, the magnitude of citrate reabsorption is much less than glucose reabsorption in the intact tubule (both substrates studied in concentrations exceeding their respective K_m) [18]. In the intact tubules, citrate reabsorption was more than 10 times higher in the proximal convoluted tubule than in the proximal straight tubule (3.4 and 0.24 picomol/mm/min, respectively). Also in the intact proximal convoluted tubule, citrate reabsorption was found to be essentially a unidirectional process, with no secretion of citrate [18]. Citrate reabsorption in the intact tubule is also consistent with predominant transport of the divalent species, based on a marked dependence on luminal pH as discussed in more detail below [26]. Although the K_m for citrate was not evaluated in the studies of intact rabbit proximal tubules, reabsorption of citrate at pH 7.4 was saturated at 1 mM luminal citrate, consistent with a K_m much below 1 mM [18].

Dicarboxylate transport in the rat proximal tubule in vivo has also been studied, using efflux of radiolabeled 2-oxoglutarate (α-ketoglutarate) as a marker [27]. Citrate inhibited 2-oxoglutarate transport (K_i = 0.2 mM at pH 6.8) consistent with citrate

transport on the same transporter as the dicarboxylate. Inhibition was predominantly via divalent citrate since inhibition was much greater at pH 6.8 than at pH 7.7.[1] These studies also demonstrated that dicarboxylate transport was predominantly sodium dependent, with a small (approximately 20%) sodium independent reabsorption [27]. The kinetics and structural specificity of dicarboxylate transport in the rat proximal tubule in vivo appear to be very similar to that in rabbit brush border membrane vesicles, consistent with a single transport system responsible for citrate reabsorption in the various species [27]. Several factors are known to alter citrate transport across the luminal membrane of the proximal tubule (for example, acid-base status, lithium, and starvation) and will be discussed below.

Peritubular uptake of citrate

Numerous whole kidney studies demonstrate net peritubular uptake of citrate, evidenced by whole kidney citrate uptake exceeding reabsorption of filtered citrate [1, 12]. However, few studies have explicitly addressed either citrate or dicarboxylate transport across the basolateral membrane of renal cells. One study did explicitly address citrate uptake in basolateral membrane vesicles isolated from rabbit renal proximal tubules and found that basolateral transport of citrate was sodium-dependent but electroneutral, in contrast to the luminal transporter [29]. The apparent affinity for citrate was also much lower (K_m of approximately 2.5 mM) than in the luminal membrane. Interestingly, and again in contrast to the luminal membrane, transport of citrate in basolateral vesicles was relatively insensitive to pH [29]. The combination of the findings of insensitivity to pH and electroneutral transport suggests that citrate transport across the basolateral membrane may be a coupled transport of one $citrate^{-3}$ and 3 Na^+ [29]. As seen in Figure 1, $citrate^{-3}$ concentrations do not change dramatically with small alterations in pH in the physiologic pH range.

Dicarboxylate transport in basolateral membranes from rat kidney has also been examined (using methylsuccinate as a substrate). Citrate inhibited methylsuccinate uptake by 60%, indicating interaction with this dicarboxylate transport system [30]. However, these studies [30] did not definitively indicate that citrate is transported to a large extent on this basolateral dicarboxylate transport system, because neither citrate transport nor the detailed interaction of citrate with methylsuccinate transport were examined. In other words, citrate may be transported across the basolateral membrane predominantly by a separate tricarboxylate carrier, but also have some interaction with the basolateral dicarboxylate transporter. An in situ study of the proximal tubule of the rat also demonstrated that citrate inhibits basolateral dicarboxylate transport in a manner similar to that in the luminal membrane [31]. However, again this does not necessarily indicate that this is the predominant pathway for peritubular uptake of citrate. A recent study (**Note added in proof**) also examined succinate and citrate uptake into rabbit basolateral membrane vesicles and confirmed several of these findings. In these studies, citrate inhibition of succinate (or dicarboxylate) transport was significantly less in basolateral membranes than in brush border membranes (**Note added in proof**). The basolateral transporter(s) for dicarboxylates resembles the luminal transporter in lithium sensitivity and structural specificity but differs in its pH dependence and its inducibility by starvation [32]. Citrate inhibition of the basolateral dicarboxylate transporter is more pronounced at alkaline pH than at acid pH, just the opposite from the luminal dicarboxylate transporter [31]. The basolateral membrane is not induced by starvation in contrast to the luminal transport mechanism. Citrate, in contrast to some of the dicarboxylates, does not appear to interact significantly with the basolateral PAH transporter or the basolateral sulfate transporter [33]. In sum, few studies have addressed the basolateral transport of citrate per se and consequently the mechanisms have not been adequately defined. Since basolateral uptake represents a significant component of total renal uptake of citrate, this is an area of needed work in the future.

Although filtered citrate is reabsorbed in the proximal tubule, the basolateral uptake of citrate might allow citrate secretion in the late proximal tubule. However, under most circumstances, whole animal studies have not been able to provide evidence for citrate secretion. However, during malate infusion and during infusion of ^{14}C-labeled precursors of citrate, evidence for citrate secretion can be demonstrated [12, 21], and also dicarboxylate secretion has been measured in untreated rats [31]. In any case, increases in urinary levels of citrate under most physiologic circumstances probably represent a decrease in reabsorption of filtered citrate rather than an increase in secretion of citrate. For instance, in alkalosis there is no evidence for increased peritubular transport into cells and, in fact, whole kidney citrate utilization is decreased [12]. No citrate secretion could be demonstrated in studies of isolated perfused proximal convoluted tubules from rabbits [18].

Alterations in citrate transport

A variety of factors have been shown to alter urinary citrate excretion [reviewed in 1]. Many of these factors have been thought to act predominantly via alterations in intracellular citrate metabolism [1]. However, many of these factors have not been studied adequately to distinguish effects on renal metabolism from effects on various transport pathways for citrate. For instance, a variety of organic acids and metabolic inhibitors increase urinary citrate and clearly increase renal citrate concentrations due to changes in metabolism, and hence may decrease citrate reabsorption via a decreased lumen-to-cell citrate concentration ratio [1]. However, some of these same organic acids and metabolic inhibitors may directly interact with citrate transport [24]. Studies to separate components of the metabolic and transport inhibition have not been performed. The focus here will be on the transport effects of a limited number of pathologic conditions or factors which have been shown to alter citrate excretion in a significant manner.

Acid-base derangements

Changes in acid-base balance have been known for decades to induce marked changes in urinary citrate [1, 19, 26]. In fact, acid-base balance appears to be the most important determinant of urinary citrate excretion. Systemic alkalosis or alkali loading

[1] However, at least one study using the rat proximal tubule in vivo has suggested that the trivalent form of citrate might be transported, based on the fact that citrate transport did not appear to be electrogenic [28].

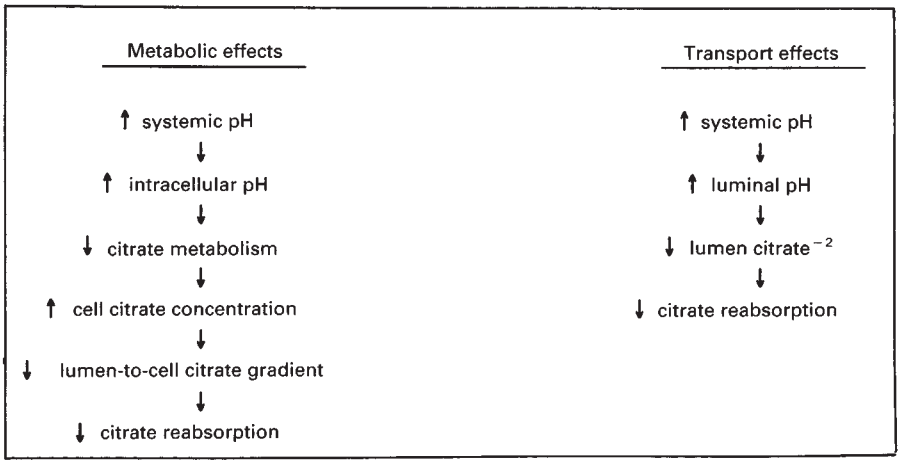

Fig. 3. *Potential mechanisms of increased urinary citrate with systemic alkalosis.* The contribution of these two types of effects to the increase in urinary citrate is discussed in the text. The opposite effects would occur with acidosis.

cause increases in urinary citrate. Acidosis or acid loads cause decreased urinary citrate excretion. The effect with acidosis is not as marked in most experimental animals since the fractional excretion of citrate is normally low even at normal pH in these species. However in humans, the fractional excretion of citrate is 10 to 35% and decreases in citrate excretion with acid loads or acidosis can be marked. Patients with renal tubular acidosis (RTA), particularly distal RTA associated with nephrocalcinosis, are well known to have reduced urinary excretion of citrate [34–36]. The changes with acidosis may be particularly germane to calcium nephrolithiasis as discussed below. Numerous experimental studies have addressed the mechanisms whereby changes in acid-base balance alter urinary citrate excretion. The discussion here will focus on recent studies which examine direct effects on transport; the details of changes in renal metabolism (which alter citrate excretion) with acid-base derangements have been reviewed elsewhere [1, 10, 14].

Changes in renal metabolism have been proposed to cause the changes in citrate excretion with acid-base imbalances [1]. Renal cortical citrate levels fall with acidosis and rise with alkalosis [17, 19]. Also, with systemic alkalosis, increasing intracellular pH and bicarbonate concentration inhibit renal cell citrate oxidation by decreasing citrate entry into mitochondria on the tricarboxylate carrier of the mitochondrial membrane [1, 37]. This results from a decreased mitochondrial pH gradient as intracellular pH rises. The decreased mitochondrial transport and oxidation of citrate with alkaline intracellular pH results in increases in intracellular citrate concentration. The opposite sequence of events occurs with acidosis. These observations have prompted the hypothesis that the metabolic changes and the subsequent changes in cellular citrate concentrations are the direct cause of changes in citrate reabsorption with changes in pH [1]. Changes in systemic pH would alter intercellular pH, resulting in changes in intracellular citrate metabolism which then alter citrate reabsorption and hence citrate excretion in the urine (Fig. 3). This proposed mechanism has been widely accepted. What is lacking in this proposal is proof that the changes in intracellular citrate alter citrate reabsorption. In fact, some evidence to the contrary has been provided. Fluoroacetate, which raises renal citrate levels, fails to alter citrate excretion [38]. Also, a recent study of the isolated perfused kidney disassociates renal citrate content from citrate reabsorption [39]. In these studies [39], citrate reabsorption and renal cell content of citrate could be disassociated during changes in plasma pH, consistent with changes in citrate reabsorption being independent of changes in renal metabolism.[2]

An additional or alternative mechanism for the changes in citrate reabsorption with acid-base derangements has been provided by studies with proximal tubule brush border membrane vesicles. Citrate uptake into brush border membrane vesicles increases with decreasing pH [25, 29, 40, 41]. These studies suggest that this pH sensitivity is due to changes in the concentration of the various ionic species of citrate with pH: citrate^{-2}, the transported species, increases with decreasing pH. The pH sensitivity of brush border vesicle citrate transport does not appear to result from proton gradients or from changes in the transporter per se. The alterations in citrate uptake in membrane vesicles obviously do not depend on cellular metabolism which is eliminated in this preparation. These findings would suggest the following scheme. Alkalosis first leads to an increased luminal pH, both from an increased filtered bicarbonate concentration, and from decreased proximal tubule proton secretion. The increased luminal pH would decrease citrate^{-2} concentrations which would then be the direct cause of decreased citrate^{-2} reabsorption (Fig. 3). Acidosis would cause the opposite changes. A testable feature of this scheme which differentiates it from the metabolic effects described above is whether luminal or cellular pH is the predominant determinant of changes in citrate reabsorption.

Recent studies of ours directly addressed the above two mechanisms by examining changes in citrate reabsorption with changes in solution pH in isolated perfused rabbit proximal convoluted tubules [26]. Solution pH was changed in either the luminal fluid or the peritubular bathing fluid by changing the bicarbonate concentration with constant pCO_2. In these experiments, decreasing peritubular pH from 7.4 to 7.2 caused an increase in citrate reabsorption. Raising peritubular pH from

[2] Also in these studies the increased renal cell citrate concentration was shown to derive from precursors of citrate, not from uptake of extracellular citrate [39].

7.4 to 7.6 resulted in a fall in citrate reabsorption, consistent with the changes observed in vivo. More importantly, the effect of luminal pH on citrate reabsorption was examined directly in a circumstance where peritubular pH was maintained constant. Decreasing luminal pH from 7.4 to 7.2 increased citrate reabsorption and increasing perfusate pH to 7.6 decreased citrate reabsorption. (In these in vitro tubule studies, the changes with alkaline solutions were statistically insignificant.) Since luminal pH has little effect on intracellular pH in the proximal tubule [42], these data [26] demonstrate that the luminal pH in the proximal tubule is an important direct determinant of alterations in citrate reabsorption with acid-base disorders. These effects of luminal pH are probably secondary to changes in the concentration of the transported ionic species, $citrate^{-2}$. In additional experiments, peritubular pH was decreased while changes in luminal pH were minimized using a perfusate which contained supraphysiologic buffering capacity. In these experiments, decreasing peritubular pH did not alter citrate reabsorption, providing additional evidence of the importance of luminal factors in the effects of acid-base disorders on citrate excretion [26]. These results have been extended by Brennan et al in experiments which demonstrated that changes in peritubular pH had no additional effect on citrate reabsorption beyond that seen with changes in luminal pH alone, again consistent with the importance of luminal pH in determining citrate reabsorption [43]. In other experiments, amiloride in the proximal tubule lumen decreased citrate reabsorption, probably secondary to inhibition of luminal acidification [44]. Thus in sum, these results demonstrate the importance of luminal pH in alterations in citrate reabsorption with changes in acid-base balance. These experiments do not exclude an additional component from the metabolic changes discussed above. In fact, both mechanisms may be valid and complementary.

An additional feature of the effect of chronic acid-base changes on citrate reabsorption has been demonstrated in recent studies. Jenkins and colleagues [45] demonstrated that chronic acid loading resulted in brush border membrane vesicles which had enhanced citrate transport compared to control rats. Chronic alkali loading did not alter citrate transport. Thus, chronic adaptations in the brush border membrane transporter may represent an additional mechanism whereby urinary citrate is decreased in the setting of chronic acidosis.

Acetazolamide

Acetazolamide, an inhibitor of carbonic anhydrase, has been recognized for many years to decrease urinary citrate excretion and thereby occasionally cause calcium nephrolithiasis [46, 47]. In whole animals, acetazolamide causes an increase in urinary citrate during the first hour followed by a decrease in urinary citrate by the second hour [19]. Kidney levels of citrate are decreased after three hours of acetazolamide [19]. The mechanism of decreased urinary citrate involves the metabolic acidosis resulting from acetazolamide; a decrease in urinary citrate is prevented if acidosis is avoided [48, 49]. Also, acetazolamide has no effect on citrate reabsorption in isolated perfused proximal convoluted tubules where luminal and peritubular pH are controlled [26].

Since acetazolamide causes urinary alkalinization despite a systemic metabolic acidosis, some investigators have concluded that urinary citrate excretion is unrelated to urine or tubular fluid pH or bicarbonate [1, 50]. According to this view, the systemic acidosis caused by acetazolamide would cause the reduced urinary citrate by reducing intracellular pH. However, despite the urinary alkalinization, a fall in proximal tubule lumen pH may still be an important component of the mechanism of the decreased urinary citrate with acetazolamide. Luminal pH may be low during treatment with acetazolamide due to a low plasma and glomerular filtrate concentration of bicarbonate, and also secondary to an acid disequilibrium pH in the lumen of the proximal tubule secondary to the inhibition of luminal carbonic anhydrase by acetazolamide. Consequently, acetazolamide may cause decreased urinary citrate secondary to decreased proximal tubule luminal pH in situ.

Potassium depletion

Potassium depletion is well known to decrease urinary citrate excretion [10, 51–53]. Potassium depletion also blocks the increase in urinary citrate excretion which occurs with systemic alkalosis [10, 51]. These effects of potassium depletion may be secondary to decreased intracellular pH [54] and/or increased H^+ secretion during potassium depletion.

Divalent cations

Several studies in the past have shown an association between increased urinary calcium levels and increased urinary citrate [for example, 55, 56]. Increases in urinary magnesium may also increase urinary citrate concentrations [8]. (Also, increasing plasma and urine citrate may increase urinary calcium excretion [57, 58].) Although the relationship between calcium and citrate is undoubtedly complex, one important factor is undoubtedly the formation of complexes between citrate and calcium in both plasma and urine [6, 7]. One study of isolated brush border membrane vesicles has shown that calcium influences citrate transport in a manner consistent with the formation of complexes between calcium and citrate [25]. At low concentrations of total citrate (physiologically relevant), added calcium complexed with divalent citrate, reducing the amount of citrate transport. Such effects have not been tested in intact tubules where other more complex interactions may occur.

Starvation

Starvation reduces urinary citrate excretion. Plasma levels and filtered loads of citrate decreases in some species but may increase in humans [59–61]. In vivo studies have demonstrated that there is also increased dicarboxylate reabsorption in the proximal tubules of starved rats [27]. Apparently the basolateral dicarboxylate transporter (which may or may not transport citrate as discussed above) is not enhanced in starvation [32]. Recently Windus, Cohn and Heifets have extended the studies of starvation to demonstrate that brush border membrane vesicles isolated from starved rats have increased citrate transport consistent with an adaptive increase in the number of citrate transporters present on the luminal membrane during starvation [61]. This represents one of the circumstances whereby an in vivo maneuver results in a chronic intrinsic alteration in the apparent number of transporters present in the apical membrane of the proximal tubule.

Lithium

Lithium (even in therapeutic doses) has been known to increase the urinary excretion of citrate and other Krebs cycle intermediates in experimental animals [62]. In man, however, therapeutic doses of lithium cause increased excretion of some Krebs cycle intermediates such as α-ketoglutarate but in general does not increase urinary citrate, perhaps suggesting some species differences in citrate reabsorption in the kidney [63]. The mechanism of these effects of lithium have been demonstrated to be secondary, at least in part, to inhibition of the dicarboxylate transporter in the brush border membrane. Lithium both competes with sodium and has non-competitive inhibitory effects on the transporter [64]. Lithium has also been shown to interact with the basolateral dicarboxylate transporter with nearly the same potency [30, 31].

Consequences of changes in urinary citrate excretion

Calcium nephrolithiasis

Calcium nephrolithiasis is the most important potential consequence of a decrease in urinary citrate. A relationship between urinary citrate, urinary calcium and stone formation has been postulated for decades [55, 65]. The details of the relationship between urinary citrate and the formation of calcium stones has recently been reviewed [4]. Briefly, citrate complexes with calcium causing a reduction in ionic calcium concentration and hence a reduction in the saturation of calcium oxalate and calcium phosphate. A significant percentage of urinary calcium may be bound by citrate [46]. Citrate directly inhibits the spontaneous nucleation of calcium oxalate and brushite [4, 66] and also inhibits crystal growth [67]. Numerous studies have shown that patients with calcium nephrolithiasis have an increased incidence (19 to 63%) of low urinary citrate [4, 5, 68, 69, 70, 71]. Many of these patients have distal renal tubular acidosis, chronic diarrhea or malabsorption leading to metabolic acidosis or low urinary magnesium [8, 68], thiazide therapy with or without potassium deficiency [72, 73] or metabolic acidosis from acetazolamide as discussed above. Also, diets rich in animal protein may be associated with low urine pH and low urinary citrate [74]. However, some patients appear to have low urinary citrate without these associated abnormalities [68, 75]. The mechanism of this abnormality is not known, but decreased intestinal absorption has been suggested [9].

Conversely, administration of oral citrate, usually in the form of potassium citrate, is used to increase urinary citrate concentrations [4]. Oral citrate has been shown to be effective in a variety of types of nephrolithiasis. Potassium citrate has the advantage over sodium citrate of also decreasing urinary calcium [76]. Most of administered citrate is probably metabolized to bicarbonate and the effect of oral citrate is the same as the administration of another alkaline load such as bicarbonate. Citrate and bicarbonate have been shown to have equivalent effects on net acid excretion and plasma bicarbonate [77]. However, some of oral citrate may be absorbed and excreted in the urine without undergoing metabolism. In considering the use of oral citrate, clinicians should note that precautions have recently been proposed against the indiscriminate administration of citrate-containing salts, especially in patients with renal insufficiency; citrate apparently causes increased aluminum absorption during simultaneous administration [78].

Acid-base balance

Not only does exogenous citrate alkalinize the urine, but endogenous citrate excretion has some significance for acid-base balance, at least in certain situations [79]. Total organic anion excretion changes significantly with changes in dietary acid and base load, and citrate represents the predominant urinary organic anion in most species [80]. Urinary citrate excretion represents a loss of potential base, an anion that can be metabolized to bicarbonate. Cooke et al [81] studied the repair of potassium depletion metabolic alkalosis in the rat with the administration of potassium bicarbonate. The correction of the alkalosis could be attributed in large part to citrate excretion in the urine [81]. More recently Kaufman, Brod-Miller and Kahn [3] studied diuretic-induced metabolic alkalosis in the rat and found that citrate excretion in the urine increased in proportion to increases in urinary net acid excretion and appeared to partially prevent a further rise in plasma bicarbonate. Kaufman and Kahn [82] have recently extended these studies to show a complementary role of citrate and bicarbonate excretion in animals with chloride and potassium depletion. Therefore, during metabolic alkalosis in the rat, increased urinary citrate may represent an important component of net acid balance. In humans, citrate excretion probably does not play the same role in acid-base balance. Although urinary citrate excretion increases with metabolic alkalosis in humans, the quantitative significance for acid-base balance is not equivalent to that in the rat [51]. Also, the correction of metabolic alkalosis in humans can be accounted for by changes in bicarbonate excretion and urinary net acid excretion, and does not require the excretion of citrate.

Conclusion

Citrate is an interesting and important urinary organic anion from many perspectives. Citrate is a significant substrate for renal metabolism. In addition, the transport of citrate in the kidney is unique: a minority ionic species is the form of citrate that is actively transported across the proximal tubule apical membrane. This latter phenomena also leads to an exquisite sensitivity of transport to changes in pH. Changes in acid-base homeostasis and other clinical derangements lead to important changes in urinary citrate. These changes can be understood (at least in part) on the basis of the known properties of citrate transport and metabolism. These changes in urinary citrate are important because urinary citrate is an important endogenous inhibitor of calcium nephrolithiasis. Urinary citrate also contributes to whole animal acid-base balance in some species.

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Note added in proof

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