Review Article

Current update and future directions on gut microbiome and nephrolithiasis

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ABSTRACT

The incidence of nephrolithiasis is increasing worldwide. Understanding how gut microbiome influences oxalate homeostasis has the potential to offer new strategies to prevent nephrolithiasis. The literature was reviewed to gather the evidence on the association between gut microbiome, hyperoxaluria and nephrolithiasis, and to identify the therapeutic interventions focused on the gut microbiome that could decrease hyperoxaluria and prevent nephrolithiasis. Gut microbiome is constituted by a plethora of microbiota including *Oxalobacter formigenes* (Oxf) and lactobacilli. Oxf can degrade dietary oxalate and induce enteral oxalate secretion. Animal studies suggested an association between oral *Oxf* supplementation and a decrease in hyperoxaluria. However, human studies have showed inconsistent results. Oral supplementation of lactobacilli did not show benefit in decreasing the hyperoxaluria. Antibiotic exposure, by affecting the gut microbiome, has been associated with an increase in nephrolithiasis. *In vivo* studies suggest fecal transplantation as a potential treatment option for reducing nephrolithiasis, but needs further evaluation in clinical studies. The current evidence suggests an association between gut microbiome and nephrolithiasis. However, the strategies focused on modulating gut microbiome for decreasing hyperoxaluria and preventing nephrolithiasis need further research. Judicious use of antibiotics in those predisposed to nephrolithiasis offers a preventative strategy for decreasing nephrolithiasis.

INTRODUCTION

In the recent National Health and Nutrition Examination Survey (NHANES) from 2007 to 2010, based on a study sample of 12,110 participants, kidney stones were found to affect approximately 1 in 11 people in the USA. The overall prevalence of kidney stones was 8.8%, with a higher prevalence in men than in women (10.6% vs. 7.1%).[1] These data represent a marked increase in the incidence of stone disease compared with the NHANES III cohort. As the NHANES data are based on the self-reported history of kidney stones, the actual prevalence may be underestimated, as some stones may be asymptomatic. The pediatric population has not escaped the stone epidemic either.[2] The Rochester Epidemiology Project[3] reported a dramatic increase in the incidence of kidney stones in children under 18 years of age during the 25-year period from 1984 to 2008. The incidence rate increased by 4% per calendar year throughout the 25-year period. The increase in the incidence was from 13/100,000 person-years between 1984 and 1990 to 36/100,000 person-years between 2003 and 2008.

FACTORS CAUSING NEPHROLITHIASIS

Metabolic abnormalities such as hypercalciuria, hypocitraturia, hyperuricosuria, hyperoxaluria, and cystinuria are the commonly identifiable etiologies of nephrolithiasis.[2,4] However, in almost 50% of the patients, no cause is found. These patients can also develop recurrent renal stones. Twin studies have revealed a 56% heritability

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of the risk for stones, while the other implicated factors include diet, exercise, work environment, and geography.[5,6] In the absence of an identifiable metabolic factor, increasing fluid intake, restricting salt, and animal protein intake are the commonly recommended preventative strategies.[7-10]

Lately, the understanding of the relationship between gut microbiome and nephrolithiasis has provided new insights into the pathways for preventing kidney stones. In the literature, the terms "microbiome" and "microbiota" are sometimes interchangeably used. Although synonymous, there is a subtle difference between these two terms. The term "microbiome" is more inclusive as it refers to the microorganisms and their metabolites, cell components, and genes.[11,12] In contrast, "microbiota" refers to the microorganisms including bacteria, viruses, fungi, and archaea, whereas the genes of the microorganisms are referenced as "metagenome." The current understanding on the microbiota has largely been derived from 16S rRNA and metagenomics.[13] Human microbiota is constituted by over 400 species of bacteria including *Oxalobacter formigenes* (Oxf), *Bifidobacterium, Lactobacillus,* and the other species of importance to the oxalate metabolism.

As 70%–80% of the kidney stones contain calcium oxalate, the regulation of oxalate homeostasis by gut microbiome to reduce the hyperoxaluria has important pathophysiologic implications. The molar oxalate-to-calcium ratio is normally at 1:10 and thus even a slight change in the urinary oxalate concentration exerts a much larger effect on the crystallization and stone formation than the comparable changes in the calcium concentration. This review focuses on the association between oxalate homeostasis and nephrolithiasis, the evidence on the role of gut microbiome in decreasing urinary oxalate, and the impact of therapeutic interventions focusing on the gut microbiome for decreasing hyperoxaluria and preventing nephrolithiasis.

MECHANISTIC PATHWAYS CONTROLLING OXALATE HOMEOSTASIS

Hyperoxaluria is considered to be a major risk factor for calcium oxalate nephrolithiasis, which occurs in about 12% of the population.[14] Oxalate homeostasis in the humans is mediated by hepatic oxalate production,[15] gastrointestinal factors, and renal oxalate excretion. Genetic defects can increase the hepatic oxalate generation, and are classified as primary hyperoxaluria (PH).[15] There are three different types of PH.[2] Dietary oxalate also contributes to the oxalate load, although the bioavailability of dietary oxalate is typically about 10% or less.[16] The intestinal oxalate absorption was initially thought to be a passive, gradient-driven flux through the paracellular pathways.[17] However, later studies established it as an active energy-dependent absorption.[18] Dietary oxalate absorption can exhibit an inter-individual variation,[16] with a higher absorption in the stone formers.[19] Dietary calcium and fats can also modify the dietary oxalate absorption.[20] It is thought that fatty acids bind calcium, and thus increase the availability of unbound anionic oxalate, making the patients with fat malabsorption predisposed to enteric hyperoxaluria. The identification of the solute-linked carrier (SLC) gene superfamily, which encodes for the proteins that mediate anion transport, opened up a new mechanistic understanding of the oxalate homeostasis.[21,22] Among the SLC gene superfamily, SLC26 gene family comprises of anion transporters expressed in the intestine with measurable affinity for oxalate.[21,22] The SLC26 gene family contributes to the oxalate homeostasis by increasing the colonic oxalate secretion, also termed as enteral oxalate shunting. It has been demonstrated that colonic oxalate secretion can serve as an extrarenal route for oxalate excretion both in rats with chronic kidney disease and in rats challenged with an oxalate load.[23,24]

With the understanding of the gut-mediated oxalate absorption and secretion, the role of gut microbiota in modulating the oxalate handling has become a renewed focus of research. The demonstration of a direct interaction between *Oxf* and colonic epithelium via colonic oxalate transporters of the SLC26 family, leading to an active secretion of endogenously produced oxalate into the gut, provided an important insight into *Oxf*-mediated oxalate elimination.[25,26] The characterization of *Oxf*-produced secretagogue further expanded on the mechanistic understanding of *Oxf*-mediated oxalate elimination.[27]

GUT MICROBIOME AND ITS DEMOGRAPHIC VARIABILITY

The microbiome has been viewed as a "metabolic organ" which provides beneficial metabolic and immunologic functions.[28] Changes in the gut microbiome have been associated with an increase in the incidence of diseases such as obesity, coronary vascular disease, allergies, and metabolic syndrome.[29] The discovery of an oxalate-degrading bacteria, *Oxf,* by Allison *et al*. in 1985 has attracted considerable attention regarding its involvement in calcium oxalate stone disease.[30] Recurrent stone formers demonstrated lower *Oxf* gut colonization rates than the first-time stone formers.[31-33] In these studies, gut colonization rate was estimated by the proportion of individuals who tested positive for *Oxf* in the stool samples. Oxf is a unique Gram-negative intestinal anaerobe that utilizes oxalate as its sole carbon and energy source and metabolizes it into formate, the anion derived from formic acid, and CO_2.[34] For the oxalate degradation, Oxf employs multiple oxalate-degrading enzymes such as oxalate oxidase, oxalate decarboxylase, oxalyl-CoA decarboxylase, and formyl-CoA transferase.[35]

With the possibility that the gut colonization of Oxf can potentially play an important role in oxalate handling, the demographics of *Oxf* gut colonization was evaluated. Gut colonization of *Oxf* has varied in studies from different

countries. In healthy adults, *Oxf* prevalence was found to be 31%–38% in the USA,[36,37] with higher colonization rates of 60%–77% in the Asian countries[38,39] and native communities.[37,40] The *Oxf* colonization rates were about two times higher in the mothers and infants for the first 3 years of life from indigenous populations living in remote areas from Venezuela and Tanzania, with limited access to modern medical practices, than their American counterparts.[41] Younger age and male gender were often found to have lower gut *Oxf* colonization rates.[37] Age-related variation was noticed, as the infants under the age of 1 year have been found to be deficient in *Oxf* colonization, whereas the colonization rate reached 90% by the age of 2 years. Nearly all the children appeared to be colonized by the age of 8 years, after which the rates declined.[42] Lack of early-life *Oxf* colonization favored a horizontal transmission route and the role of environmental factors rather than maternal transmission.[41,42] A higher *Oxf* colonization in the indigenous population as compared to the general population exposed to the typical medical practices such as antibiotic usage has been suggestive of an adverse role of modern medical practices on *Oxf* colonization.[41]

EVIDENCE ON THE ROLE OF *OXALOBACTER FORMIGENES* IN DECREASING HYPEROXALURIA

With the understanding that gut plays an important role in the oxalate homeostasis, various gut-focused intervention strategies to reduce the hyperoxaluria were evaluated. Reducing the consumption of oxalate-rich foods can be an obvious intervention; however, the long-term effectiveness and undesirable consequences of such dietary limitations remain uncertain. Therefore, the role of *Oxf* supplementation for decreasing the hyperoxaluria was explored. Gut colonization of the noncolonized rats by using either the oxalate-degrading enzymes from *Oxf*[32] or the viable whole *Oxf* cells reduced the urinary oxalate excretion.[32] In the mouse model of PH type 1, which is deficient in liver alanine-glyoxylate aminotransferase (agxt) that leads to an increase in the endogenous hepatic oxalate production and hyperoxaluria, *Oxf* feeding resulted in a striking reduction in hyperoxaluria.[43,44] The reduction in the hyperoxaluria in these studies was thought to be mediated by a favorable transepithelial gradient created by the *Oxf*-induced dietary oxalate degradation and the *Oxf* interacting with oxalate transporters of the SLC26 family.[25-27]

In human stone formers, the absence of intestinal *Oxf* colonization correlated with an increased risk of hyperoxaluria.[31,45] The demonstration of similar intestinal oxalate absorption in stone formers with hyperoxaluria,[31] indirectly supported the enteral oxalate secretion in humans similar to that reported in the mouse model.[25] Although several studies have linked the absence of *Oxf* in gut to the higher urinary oxalate excretion,[45-47] these findings were not uniformly replicated.[36,48]

With this baseline evidence on the role of *Oxf* in reducing the hyperoxaluria, translational human studies were conducted. Oral *Oxf* administration was tried as a bridging procedure until transplant in two infants with infantile oxalosis with end-stage renal disease.[49] In both the infants, *Oxf* was administered twice a day for 4 weeks and the therapy reduced the plasma oxalate levels by about 50%, despite no concomitant change in the dialysis prescription. The treatment was well tolerated.[49] The end-stage renal status in both the infants supported that the *Oxf* therapy improved the intestinal secretion of endogenous oxalate.[50] Subsequently, a Phase I study demonstrated a statistically significant reduction in urinary oxalate excretion over 4 weeks by the oral administration of an *Oxf* formulation (Oxabact®, OC3a).[51] However, a larger trial using a modified formulation (OC3b) failed to demonstrate a decrease in urinary oxalate excretion over a period of 24 weeks.[49] The lack of benefit in this trial was thought to be due to lyophilized state of the OC3b formulation, leading to its lower bioactivity and longer latent period to achieve peak oxalate-degrading capacity. As a result, the formulation was modified, which resulted in the use of the OC5 formulation in the subsequent trial.[52] Unfortunately, twice-daily treatment with the OC5 formulation over an 8-week treatment period in patients with PH again failed to show a decrease in the urinary oxalate excretion.[52]

The negative results in both the studies, despite the demonstration of adequate *Oxf* gut colonization, questioned the biologic effectiveness of *Oxf* supplementation in humans for treating PH. However, these studies also suggested the need to revisit the bioavailability of the different *Oxf* preparations. Methodological issues may also have affected the results. Despite the negative results based on the 24-h urinary oxalate measurements, urine oxalate normalized for urinary creatinine differed between the patient and the control arms.[49] This measurement discrepancy suggested the possibility of an inadequate 24-h urine collection, which is known to be a challenge in children. Moreover, urinary oxalate excretion exhibits a large intra-individual variability.[53] Patient's estimated glomerular filtration rate can also influence the urinary and serum oxalate levels.[49,52] The treatment duration in these studies might have been insufficient to demonstrate treatment effectiveness.[49,52]

Unlike the mouse model, where the confounders are controlled for, the effectiveness of *Oxf* in humans can be influenced by diet and other factors as well. In one study, urinary oxalate excretion was significantly lower in *Oxf*-positive patients on a standardized diet as compared to a self-selected diet.[31] *Oxf* colonization decreased the urinary oxalate excretion more effectively during the periods of low calcium and moderate oxalate intake,[54] suggesting the possibility of reduced effectiveness of *Oxf*

supplementation when dietary oxalate is restricted.[55,56] However, liberalizing dietary oxalate can in turn increase urinary oxalate levels by increasing the intestinal oxalate absorption.[57] Based on the evidence so far, further research is needed to establish the effectiveness of *Oxf* supplementation as a therapeutic tool to prevent nephrolithiasis in a clinical setting.

EFFECTIVENESS OF *LACTOBACILLUS* SPECIES FOR PREVENTING NEPHROLITHIASIS

Probiotics containing lactobacilli have been commonly used to treat gastrointestinal symptoms such as antibiotic-induced diarrhea. Therefore, probiotics containing lactobacilli were also considered as a potential treatment option for treating hyperoxaluria. A *Lactobacillus* preparation containing *Lactobacillus acidophilus, Lactobacillus brevis, Streptococcus thermophilus*, and *Bifidobacterium infantis* named Oxadrop® was formulated specifically for treating hyperoxaluria.[58] Initial uncontrolled and unblinded studies showed a favorable response to Oxadrop® in humans with a 40% drop in oxalate excretion in a group of mildly hyperoxaluric calcium-oxalate stone formers.[58,59] In addition, a dose–response pattern was assessed by administering Oxadrop® at 4, 8, and 12 g doses for 1 month each.[58] These data suggested a dose–response association, with a small effect at 4 and 8 g, whereas the 12 g of Oxadrop® resulted in a 20%–25% decrease in the urinary oxalate excretion and attained a urine oxalate level close to the baseline.[58] Based on these positive results, a randomized, placebo-controlled trial in a population of forty enteric hyperoxaluria stone formers was performed.[55] In addition to Oxadrop® supplementation, patients were placed on a controlled metabolic diet with normal calcium and reduced oxalate. The diet itself was effective in reducing urine oxalate excretion by an average of 36%. However, urinary oxalate did not fall further with either probiotic or placebo while maintaining the controlled diet. Subsequently, a randomized, placebo-controlled trial of Oxadrop® with nonrestricted diet also failed to show a reduction in hyperoxaluria in twenty stone formers with idiopathic mild hyperoxaluria.[60] The investigators then considered if oxalate availability helps in maintaining *Oxf* colonization,[55,56] and therefore a low calcium (400 mg) and high oxalate (200 mg) diet was evaluated in a population of 14 stone formers.[57] High dietary oxalate increased urinary oxalate excretion by 30%, and addition of *Lactobacillus/Bifidobacterium* preparation had no further effect on urinary oxalate levels.[57] Thus, based on the current literature, there is no evidence that lactobacilli supplementation benefits hyperoxaluria.

ROLE OF ANTIBIOTICS IN PRESERVING GUT MICROBIOME

In addition to *Oxf* and lactobacilli supplementation for decreasing the hyperoxaluria, strategies to preserve the gut microbiome have also gained attention. In this context, the influence of antibiotic exposure on the gut microbiome was highlighted by observational studies, suggesting low *Oxf* colonization because of the antibiotic exposure in patients with cystic fibrosis[46] or inflammatory bowel diseases.[61] The detrimental effects of antibiotics on the gut *Oxf* colonization was further supported by *in vivo* studies that demonstrated the susceptibility of all the four *Oxf* strains found in humans to the commonly prescribed antibiotics.[62] The recent analysis of a large database constituted of 26,000 patients and nephrolithiasis with approximately 260,000 matched controls provided a robust epidemiological evidence on the association between antibiotics and nephrolithiasis.[63] In this analysis, the exposure to any of the five different antibiotic classes 3–12 months before the index date was associated with nephrolithiasis, though the association varied among the different antibiotics. The adjusted odds ratio (95% confidence interval) was 2.33 (2.19–2.48) for sulfas, 1.88 (1.75–2.01) for cephalosporins, 1.67 (1.54–1.81) for fluoroquinolones, 1.70 (1.55–1.88) for nitrofurantoin/methenamine, and 1.27 (1.18–1.36) for penicillins.[63] Two of the antibiotic classes, tetracyclines and macrolides, did not show any association. Although it is plausible that antibiotics increased the risk of nephrolithiasis in this study by deranging the gut microbiome, the study design precluded the assessment of a mechanistic inference or a causal relationship. However, the *in vivo* evidence supported the theory of disruption of the gut microbiome for linking antibiotic exposure to the nephrolithiasis risk.[62] In the *in vivo* study, all the four *Oxf* strains were resistant to amoxicillin, amoxicillin/clavulanate, ceftriaxone, cephalexin, and vancomycin and sensitive to azithromycin, ciprofloxacin, clarithromycin, clindamycin, doxycycline, gentamicin, levofloxacin, metronidazole, and tetracycline.[62] In a prospective small study, antibiotic exposure to either clarithromycin or metronidazole in *Helicobacter pylori*-positive group decreased the *Oxf* colonization in 38% of the patients at 1- and 6-month follow-ups in contrast to the sustained positivity in 92% of the controls who did not receive the antibiotics. The notable finding from this study was that only one of the participant who had *Oxf* colonization eliminated by antibiotics regained colonization at 6 months, suggesting a lasting impact of antibiotics on *Oxf* colonization.[64]

In clinical practice, the nonjudicious use of antibiotics can be defended by the belief that the temporarily disrupted gut microbiome will recover to normal. Although this matter remains understudied, microbiome recovery can explain a proportionately lower stone prevalence despite a widespread antibiotic use. A decline in the risk of nephrolithiasis over time after the antibiotic exposure may also support microbiome resilience or microbiome recovery.[63] However, this microbiome resilience may exhibit substantial inter-individual variability, thereby making some individuals more susceptible to nephrolithiasis after antibiotic exposure. The robustness of gut colonization may also vary among different individuals.[37] Lower gut microbiome reserve can partly explain the higher prevalence

of nephrolithiasis in males and in those with high body mass index,[37,63] although in males, lower urinary citrate excretion after puberty can be another risk factor.[65] Those exposed to multiple antibiotics such as patients with cystic fibrosis[46] or inflammatory bowel disease[61] may not get enough time in between the antibiotic exposures for the microbiome to recover.[63,64] The long-term consequences of antibiotic exposure were also supported by *in vivo* studies which linked antibiotics to systemic diseases such as obesity, diabetes, and atherosclerosis.[66-68] This inference on the long-term effect from antibiotic exposure was also supported by a population-based, case–control study that suggested an increase in the risk of Type 1 diabetes after antibiotic exposure in childhood.[69]

Although the association between the antibiotic usage and the risk of nephrolithiasis needs further evaluation, this association may have a significant clinical relevance, given the widespread antibiotic use for marginal indications. In the USA, health-care providers prescribed 262.5 million courses of antibiotics in 2011 (842 prescriptions/1000 persons).[70] Previous studies estimated that about 30% of the antibiotics prescribed in the outpatient setting in the USA are unnecessary;[71,72] the proportion could be even higher in countries with unregulated medication prescription. Based on this evidence, a judicious use of antibiotics in those predisposed to nephrolithiasis may serve as a major preventative strategy for decreasing the recurrence of nephrolithiasis.

GUT MICROBIOME PROFILING, FECAL TRANSPLANTATION, AND NEPHROLITHIASIS

The understanding on gut microbiome profiling has led to the concept of fecal transplantation or "bacteriotherapy." Fecal transplantation is the administration of a solution of fecal matter from a donor into the intestinal tract of a recipient in order to change the recipient's microbial composition so as to confer a health benefit.[73] The first known description of the use of feces as therapy was described by Ge Hong in the 4th century in China for the treatment of a variety of conditions including diarrhea.[74] In 1958, the use of fecal enemas was used for the first time in clinical medicine for the treatment of pseudomembranous colitis.[75] In a murine model, fecal transplantation of the germ-free mice with obese microbiota resulted in a significantly greater increase in adiposity than those transplanted with the lean microbiota.[76] Metabolic diseases such as asthma,[77] inflammatory bowel disease,[78] and cardiovascular diseases[79] have been linked to the changes in the gut microbiome profile. Similarly, both obesity and diabetes, the known risk factors for nephrolithiasis,[1] have been associated with unique gut microbiome profiles.[80] The effectiveness of fecal transplantation in metabolic syndrome was shown by improved insulin sensitivity in individuals with metabolic syndrome by transferring intestinal microbiota from lean donors.[81]

In clinical practice, the benefit of fecal transplantation was demonstrated in the treatment of *Clostridium difficile* infections.[82] The fecal transplantation is thought to cause a competitive exclusion of *C. difficile* for the nutrients by the transplanted microbiota.[83] The restoration of gut microbiome by fecal transplantation has been found to be sustained.[84] Fecal transplantation has also shown potential to treat inflammatory bowel disease in a clinical setting, although with inconsistent results.[83,85,86] The multifactorial pathophysiology of inflammatory bowel disease, unlike that in recurrent *C. difficile* infection, was considered to be a reason for the inconsistent results,[83] suggesting the need of disease-specific assessment while evaluating the effectiveness of fecal transplantation.

The advances in gut microbiome sequencing technology has provided new insights into the gut microbiome profiling associated with nephrolithiasis beyond the individual bacteria such as *Oxf* and lactobacilli. Gut microbiome sequencing revealed that *Bacteroides*, a genus of Gram-negative obligate anaerobic bacteria, were more abundant in the stone former group, and *Prevotella*, a different genus of Gram-negative bacteria, were more abundant in the nonstone former group.[87] In another controlled study, gut microbiome profile was found to be different in patients with and without nephrolithiasis.[88] In a metagenomics analysis, two enzymes, formyl-CoA transferase and oxalyl-CoA decarboxylase, were found to be less well represented in the stone formers as compared to the controls, which can lead to a higher oxalate availability in the gut for absorption.[89] In a mouse study, fecal transplantation from healthy mice into the germ-free mice demonstrated a significant decrease in urinary calcium, oxalate, and ammonium and an increase in urinary pH at 4 weeks following fecal transplantation.[90] Whole-gut microbiome transplanted from the oxalate-metabolizing wild mammalian herbivore, *Neotoma albigula*, to the laboratory rat, *Rattus norvegicus*, which is incapable of degrading oxalate, showed an increase in oxalate degradation in the transplanted rat that persisted 9 months after the transplant.[91]

With further understanding on gut microbiome in nephrolithiasis, there is a need to reinterpret the mechanism by which gut microbiome can influence nephrolithiasis. It can be hypothesized that targeting the gut microbiome as a whole unit, constituted by the network of microbes, can be a more effective strategy to reduce nephrolithiasis instead of focusing on individual bacteria such as *Oxf* or *Lactobacillus*. Potentially, gut microbiome transplantation, rather than *Oxf* supplementation alone, may result in sustained oxalate degradation in the human gut. In this context, restricting unnecessary antibiotic exposure offers a synergistic strategy to ensure healthy gut microbiome profile, especially in those who are predisposed to nephrolithiasis.

CONCLUSIONS

Although establishing a direct causal relationship between alterations in the gut microbiome and the risk of kidney stones is nonconclusive at this point, the reviewed literature is highly suggestive of an association. The positive results reported in the rodent studies, in which the gut microbiome was manipulated with *Oxf* supplementation, could not be replicated in human studies. Given the fact that human microbiome and diet are significantly different from an experimental rodent model, a more representative approach may be necessary in order to translate the findings from a rodent model into the humans. The methodological limitations identified in the previous studies can help in designing the future studies. The advancement in sequencing technologies and analytical tools offer an opportunity to refine the understanding on the association between gut microbiome and nephrolithiasis. In addition, looking at the gut microbiome as a network of bacterial species performing a function, for example, oxalate degradation, instead of as a single species, offers a new perspective for future research. Judicious use of antibiotics in those predisposed to nephrolithiasis should be considered as a preventative strategy to decrease nephrolithiasis.

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