Potentially beneficial approaches include renin angiotensin aldosterone system (RAAS) inhibitors. Thus the reader is also directed to recent comprehensive reviews on the subject of permeability factors in NS, detailing the soluble urokinase plasminogen activator receptor story and proposing putative factors and translational strategies (18, 59). More recently, it has been suggested that even in MCD, both a permeability factor of immune origin and podocyte-derived factors may contribute to the development of NS (reviewed in Ref. 43).

Edema is one of the classic clinical features of childhood NS and one of the principal reasons for admission to the hospital. The underlying mechanisms of edema formation remain a subject of ongoing investigation. This review aims to focus on the current understanding of edema formation in NS and to discuss recent developments in this area, potential targets for therapy, and novel areas that need further scientific exploration.

Pathogenesis of Edema in NS: Under- vs. Overfill Hypothesis

Two major opposing theories on the pathophysiological mechanisms underlying the development of edema in NS have been proposed: 1) the underfill hypothesis and 2) the overfill hypothesis (7, 13).

The underfill hypothesis. The underfill hypothesis was generated almost a century ago (27). It postulates that high-grade proteinuria results in hypoalbuminemia, leading to a reduction in plasma oncotic pressure with consequent leakage of plasma water into the interstitium, generating edema. The resultant diminished intravascular volume manifests with tachycardia, hypotension, and oliguria as well as peripheral vasconstriction and water and sodium retention. This is affected by activation of the renin-angiotensin-aldosterone system (RAAS), coupled with an increase in plasma norepinephrine and arginine vasopressin (AVP) concentrations (65, 76, 95). Together, this neurohormonal activation results in a highly concentrated urine with very low sodium content (44). In a study of 16 pediatric and adult patients with NS with normal renal function, Usherti et al. (92) found decreased plasma sodium concentration, increased plasma AVP concentration, and an elevation in plasma renin activity (PRA) and urinary norepinephrine levels, coupled with impaired excretion of an acute water load, compared with controls. They demonstrated a highly significant inverse correlation between plasma AVP concentration and blood volume in these nephrotic patients (92). Therefore, in this scenario, the renal sodium retention was thought to occur as a secondary physiological response to underfilling (a reduced effective intravascular volume) (8). The therapeutic implications of this hypothesis are clear: expansion of intravascular volume and restoration of plasma oncotic pressure by administration of a colloid such as albumin would prove beneficial. Indeed, albumin infusion-induced volume expansion in children with NS has been shown to reduce PRA,
aldosterone, and AVP and to improve glomerular filtration rate (GFR), urine output, and sodium excretion (70, 90).

However, a decrease in measured plasma volume in nephrotic patients has not been a universal finding, and in some nephrotic states plasma volume may actually be increased (24, 34, 66). Furthermore, elevated PRA or aldosterone, expected in the setting of decreased blood volume, has only been confirmed in about half of studied nephrotic subjects (35, 66). However, these variations in findings may be accounted for by the fact that these parameters are dependent on the hemodynamic status of the patients at the time of the study.

Some clinical situations also do not fit well with the underfill hypothesis. The treatment of edema in NS with albumin alone reduces PRA, but it is often insufficient to induce diuresis without the addition of a diuretic (11, 94). In contrast, some patients respond to diuretics alone without the need for albumin (44). Moreover, the reduction of the renin-aldosterone axis by mineralocorticoid receptor antagonists or angiotensin-converting enzyme inhibitors does not result in increased sodium excretion in most patients with NS (10, 94). In fact, neurohumoral markers such as renin and aldosterone levels do not show a consistent pattern, suggesting volume depletion in some, and normal or excess volume in other patients (9, 11, 52, 61, 93, 94, 96). In some patients, the administration of albumin is associated with volume overload and causes hypertension and pulmonary edema (71). To the observant physician (and parent), the earliest sign of the patient entering remission is a large diuresis, subsequently confirmed by the improvement in proteinuria. This occurs well before plasma protein levels (and thus plasma oncotic pressure) have normalized (64). Finally, there are patients with other causes of analbuminemia who do not typically suffer from edema (12, 54). Taken together, these findings suggest that the generation of edema in NS is not just secondary to a reduction in plasma oncotic pressure as a result of hypoalbuminemia caused by high-grade proteinuria.

Ichikawa et al. (42) provided strong experimental evidence against the underfill hypothesis in a rat model of nephrosis, in which they performed nephron puncture after infusing puromycin aminoglycoside (PAN) into one kidney to independently assess the effect of proteinuria in both the nephrotic and the normal kidney. They noted that only the nephrotic kidney avidly conserved sodium, despite maintaining euvoolemia by controlling plasma protein levels within the normal range by infusion of homologous rat plasma. This suggested an intrarenal mechanism of salt retention as opposed to a circulating neurohumoral factor. In a separate study, Chandra et al. (15) found that the control kidney actually increased sodium excretion, as if sensing intravascular volume excess. They showed that sodium delivery to the collecting duct was comparable in both kidneys, whereas the final sodium excretion was higher in the normal kidney, therefore suggesting that the collecting duct was the main site of avid sodium reabsorption in the nephrotic kidney.

The overfill hypothesis. Due to the inconsistent findings described above, the overfill hypothesis was proposed as an alternative explanation for the development of edema in NS: proteinuria causes primary sodium retention with consequent volume expansion and leakage of excess fluid into the interstitium (25, 61). In support of this idea, Dorhout Mees et al. (25) observed that blood pressure and plasma volume fell in patients with NS after prednisone-induced remission (25). In 88 patients with NS, Gur et al. (36, 77) reported higher plasma and blood volume corrected for estimated lean body mass compared with controls.

The Epithelial Sodium Channel and Sodium Retention in NS

What mediates the primary sodium retention? Ichikawa et al. (42) identified the collecting duct as the main segment involved in sodium retention in NS (42). Initial focus was on the basolateral Na-K-ATPase, as there is evidence from studies in rodent models of nephrosis to suggest an increase in levels and activity of Na-K-ATPase in the collecting ducts of PAN-induced nephrotic rats (20, 97). Recent evidence suggests the principal means of sodium reabsorption in NS occurs in the distal segment of the nephron via the epithelial sodium channel (ENaC) (Fig. 1) (50). Using both the PAN and HgCl2 rodent models of NS, Kim et al. (46, 47) demonstrated an increase in the expression and targeting of ENaC to the apical cell surface, which is aldosterone dependent (19, 57). Lourdel et al. (57) showed a linear correlation between plasma aldosterone and ENaC abundance. Although the prevention of hyperaldosteronemia by adrenalectomy prevented the increase in apical ENaC targeting, it did not prevent sodium retention and the development of NS in the PAN-treated rats (19, 57). Although the adrenalectomized rats did not show an increase in ENaC expression, they had a similarly low urinary sodium excretion as adrenal-intact nephrotic rats with increased ENaC expression (57). Treatment with amiloride returned sodium excretion to normal despite a lack of increase in ENaC expression (57). This aldosterone-independent sodium retention in NS by the ENaC was further illustrated by Deschenes et al. (22), who showed that sodium retention can be prevented in the PAN-induced rat by amiloride and not aldosterone inhibition.

ENaC activity depends on channel density at the apical membrane, which is aldosterone and vasopressin dependent, and on channel activity, which is regulated by proteolytic processing and by anionic phospholipids on the inner cell membrane (Fig. 1) (38, 58). The ENaC is composed of the α-, β-, and γ-subunits (74), of which the α- and γ-chains have a regulatory role. An ENaC channel with uncleaved α- and γ-chains has low open channel probability and conducts little sodium (40). Sequential proteolytic cleavage of the α- and γ-subunits results in increasing open channel probability (40). Cleavage of an inhibitory tract from the γ-subunit by serine proteases such as plasmin results in near full activation of the ENaC (14). More recently, plasmin has been directly shown to activate ENaC and promote sodium and water retention in nephrotic rodents and humans (Fig. 2) (83). The plasmin precursor, plasminogen, is filtered by the nephrotic kidney and is activated to plasmin by the tubular urokinase-type plasminogen activator present in the rat and human kidney. Use of the potassium-sparing diuretic, amiloride, resulted in increased urine sodium excretion and reduced ascites volume in the PAN nephrosis rat. These effects were attributed both to inhibition of ENaC and inhibition of urokinase-type plasminogen activator by amiloride, thus reducing the amount of active plasmin present in the urine (Fig. 2).

The dominant role of the ENaC in sodium retention and the development of nephrotic edema are underscored by the reversal of sodium retention by amiloride in animal models. It provides a rationale for specific blockade of ENaC with
amiloride for the treatment of sodium retention and edema formation in NS. While the administration of amiloride before the onset of sodium retention fully prevented sodium retention and edema formation in PAN-induced nephrotic rats (22), clinicians usually encounter patients who are well beyond this early stage, many of whom have anasarca. In this situation, treatment aims both to limit further sodium retention and to promote diuresis and natriuresis. Under normal conditions, sodium reabsorption along the collecting duct is quantitatively low, thus reducing the ability of amiloride to promote massive sodium excretion. Loop diuretics decrease sodium reabsorption along the thick ascending limb of the loop of Henle, increasing distal sodium delivery, which, in turn, overloads the sodium reabsorptive capacity of the distal nephron, resulting in natriuresis. However, nephrotic patients display increased sodium reabsorptive capacity along the cortical collecting ducts, thereby blunting the natriuretic effect. Combined use of loop diuretics and amiloride, which inhibits distal sodium reabsorption, could overcome the resistance to therapy with a loop diuretic alone. Accordingly, Deschenes et al. (21) showed that combined therapy with amiloride and furosemide resulted in greater natriuresis and weight loss than monotherapy with either agent alone in pediatric patients (21). There has been no study interrogating the effectiveness of amiloride monotherapy in childhood NS. It has to be emphasized that most of the findings in support of the overfill hypothesis are derived from animal studies with few studies in humans.

In NS, ENaC activation occurs following proteolytic processing of the protein. Therefore, inhibition of serine protease activity could represent another potential therapeutic target (82). Accordingly, camostat mesilate (CM), a synthetic inhibitor of serine proteases such as plasmin and prostatin, was shown to reduce aldosterone-induced renal ENaC proteolysis in rats (91). In a small case series, treatment with CM caused a significant reduction in proteinuria in patients with NS of various etiologies. Although these reports suggest that systemic administration of serine protease inhibitors to human patients is well tolerated, improves proteinuria, and attenuates edema formation through inhibition of ENaC-mediated sodium retention, the potential for systemic effects related to unspecific protease inhibition suggests a note of caution (82). Using inhibitors that are freely filtered across the glomerular barrier or secreted into the tubules could potentially reduce the side effects associated with systemic inhibition of protease activity (82).

The Role of Corin

Corin is an endogenous type II transmembrane serine protease that cleaves pro atrial natriuretic peptide (ANP) and pro brain natriuretic peptide (BNP) to their biologically active forms and is detected in multiple organs, including the heart, brain, and kidney as well as in the serum and urine (23, 31, 68, 102, 103). Its importance in blood pressure was demonstrated by the demonstration of salt-sensitive hypertension in corin-deficient mice (102). ANP has three important roles in sodium and water excretion in the kidney, namely, 1) inducing afferent arteriolar vasodilation and efferent vasoconstriction, thereby increasing GFR; 2) causing natriuresis through inhibition of the N+/H+ exchanger in the proximal tubule, the distal Na+/Cl− cotransporter, and ENaC; and 3) causing diuresis through inhibition of aquaporin-2 insertion in the apical membrane of
the collecting duct, normally mediated via arginine vasopressin (reviewed in Ref. 2). Polzin et al. (69) also suggested the role for corin in the salt retention in NS. They demonstrated a reduction in kidney corin expression in the PAN nephrosis rat coupled with a concomitant increase in pro-ANP and decreased ANP levels. They then proceeded to demonstrate increased amounts of renal β-ENaC and its activators (phosphodiesterase 5 and protein kinase GII) in corin knockout mice compared with wild-type mice (69). Further exploration of the link between corin and ENaC may further enhance our understanding of the salt retention in NS (48).

**The Role of Aquaretics**

In NS, vasopressin levels are reported to be elevated, leading to the production of concentrated urine and impaired excretion of a water load (44, 65, 95). In keeping with this observation, nephrotic patients are often hyponatremic, consistent with a relative water excess (92). Type 2 vasopressin receptors (AVPR2) mediate urinary concentration in the collecting duct, and AVPR2 inhibitors have proven effective in the treatment of edema (1). Konomoto et al. (51) highlighted a potential role of vasopressin in edema formation in a child with relapsing steroid-sensitive NS who developed cranial diabetes insipidus. Relapses of NS before the development of cranial diabetes insipidus and after treatment with DDAVP were associated with edema. However, when a relapse occurred in the presence of untreated cranial diabetes insipidus, the patient was edema-free despite high levels of proteinuria and low plasma albumin. In another report, a child with steroid-resistant NS and edema, pleural and pericardial effusions resistant to treatment with albumin/furosemide responded significantly to tolvaptan (a selective, competitive vasopressin receptor 2 antagonist), despite persistent nephrotic range proteinuria and unchanged plasma albumin levels (79).
While these case reports offer a novel approach to treating edema in nephrotic patients, injudicious use of aquaretics may be fraught with potential risks of serious hypovolemia, hypernatremic dehydration, shock, and thrombosis (6), especially in the consideration of the thrombophilic tendencies in NS (80). Aquaretics are, thus, best avoided in NS.

“Urearetics”: Blocking Urea Transporter Proteins

Since urea transport plays an important role in urinary concentration, urea-blocking agents may represent another therapeutic tool to treat NS-related edema (67, 72). Urea transporter (UT) proteins facilitate the passive transport of urea, driven by a concentration gradient, across cell membranes, and are essential in the urinary concentrating mechanism (28). Different isoforms of the transporters UT-A and UT-B are expressed in the descending limb and the collecting duct of the medullary nephrons (28, 45, 81, 85, 98). UT receptor accumulation in the plasma membrane is regulated mainly by vasopressin (via V2 receptors) (4, 5, 41, 49, 106) and hypertonicity (3, 99, 100). UT expressed in the inner medullary collecting duct is responsible for urea reabsorption, which maintains a hypertonic medullary interstitium. The highly urea-permeable fenestrated ascending vasa recta facilitate transfer of urea from the inner to the outer medulla. The interstitial urea from the ascending vasa recta is partially reabsorbed by the nonfenestrated descending vasa recta and the

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Fig. 3. Urea transporter blockade by “urearetics”, salt-sparing diuretics. Aquaporin and urea transporters in the descending thin limb of the loop of Henle and inner medullary collecting duct facilitate the passive transport of urea, driven by a concentration gradient, across cell membranes, and are essential in the urinary concentrating mechanism. Urea transporter inhibition and its subsequent effect on the countercurrent mechanism may be promising therapeutic targets as it has the potential to be effective in refractory edema, producing a relatively salt-sparing diuresis compared with conventional diuretics. Adapted from Ref. 33.

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thin descending limb of the loop of Henle by a countercurrent exchange mechanism in which urea recycling conserves renal urea (67, 72, 73). UT-deficient mice demonstrate a reduced capacity to concentrate urea in the urine, manifesting as a reduction in maximum urinary osmolality and up to a twofold increase in daily urine output (32, 104) (Fig. 3).

Various small-molecule inhibitors of UT isoforms have been developed and tested successfully as diuretics in different rodent models (29, 30, 55, 105). Selective UT-A isoform inhibition has an added advantage for potential diuretic therapy in that it is primarily expressed in the kidneys, reducing the potential of extrarenal effects compared with UT-B, which is relatively ubiquitous (28). These studies collectively highlight urea transporter inhibition as potential targets for therapeutic drug development. Their effect on the countercurrent mechanism may be promising as it has the potential to be effective in refractory edema, producing a relatively salt-sparing diuresis compared with conventional diuretics. Its use (with or without conventional diuretics) may be effective in hyponatremia due to the syndrome of inappropriate antidiuretic hormone secretion and in fluid-overload states like congestive cardiac failure, chronic liver failure, chronic kidney disease, and NS. However, further testing of these inhibitors in animal models, and ultimately in clinical trials, is needed to define their safety and efficacy before mainstream therapeutic use.

Alternative View on Extrarenal Sodium Balance

Conventional understanding of sodium balance maintains that extracellular body fluids readily equilibrate across compartments, that electrolyte concentrations in various compartments are constant, and that the kidney is the main player in controlling the body’s sodium content. This paradigm has been questioned by recent studies that showed “kidney-like” lymphatic and blood vessel countercurrent systems in the skin, intestines, bone, and elsewhere in the body (53, 56, 86–88). The notion that the immune system plays a role as homeostatic regulator of interstitial electrolyte homeostasis and that salt induces proinflammatory immune cell polarization has extended the role of the immune system to physiological adaptation, interstitial fluid matrix regulation, blood pressure control, and cardiovascular disease (60, 101). The protein-rich and negatively charged glycosaminoglycan-rich endothelial surface layer may act as a buffer to excess intravascular sodium, by binding and osmotically inactivating it (89), and a barrier to water entering the interstitium, which make this an intriguing area of future study for sodium hemostasis in both health and disease (reviewed in Ref. 63). These findings may have implications for our understanding of the sodium and fluid retention in NS and drive the development of entirely novel therapeutic agents.

Conclusion

Understanding the mechanism of edema formation in NS has fascinated clinicians and scientists for decades, and the study of renal physiology in the nephrotic state has shed light on normal physiology. The observation that proteases in the glomerular filtrate activate the sodium-retaining ENaC, thus promoting intrarenal salt retention, has yet to be translated into a new therapeutic approach, despite the availability of amiloride. Is this because of concerns about hyperkalemia and the potential ineffectiveness of ENaC inhibition alone, or is there more to the story? Can aquaretics and urearetics be used to manage edema in NS? These key questions need to be answered.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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