Renal and Electrolyte Disorders

Eighth Edition

Robert W. Schrier

Wolters Kluwer

Renal and Electrolyte Disorders

EIGHTH EDITION

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PREFACE

The eighth edition of *Renal and Electrolyte Disorders* continues to be an exciting challenge to update because of the many advances that have occurred in the various areas of renal pathophysiology over the past five years in the revolutionary era of biomedical science. Since the kidney is responsible for maintaining the milieu intérieur in health and disease, it is very difficult for any physician to practice state-of-the-art medicine without an up-to-date knowledge of renal physiology and pathophysiology.

For over 40 years, virtually thousands of medical students, house officers, and fellows have been introduced to the intricacies of renal physiology and pathophysiology by reading and studying *Renal and Electrolyte Disorders*. This is both a remarkable tradition and a demanding responsibility to which a brilliant group of authors have responded in the eighth edition of *Renal and Electrolyte Disorders*.

The recent developments in disorders of water homeostasis have been very exciting and are discussed by Tomas Berl and Robert Schrier. The vasopressin receptor has been cloned, as have several water channels (aquaporins) including the collecting duct water channel which responds to vasopressin. This has allowed the delineation of mutation defects causing congenital nephrogenic diabetes insipidus. Now there are nonpeptide, orally active vasopressin antagonists that are clinically available as "aquaretics," that is, drugs that increase only solute-free water, but not electrolyte, excretion. These agents can treat the hyponatremia associated with the syndrome of inappropriate antidiuretic hormone secretion (SIADH), cirrhosis, and cardiac failure. A detailed understanding of the afferent and efferent mechanisms of renal sodium retention in edematous disorders, including the optimal use of diuretics, is discussed in the context of body fluid volume regulation in health and disease.

The acid–base disorders have been updated in a lucid and understandable manner by Zeit Khitan, Joseph Shapiro, and Seth Furgeson. Biff Palmer and Thomas DuBose have added substantial new information in their potassium chapter including the advances in genetic

hypokalemic and hyperkalemic disorders.

Mordecai M. Popovtzer provides the most up-to-date information on calcium, phosphorus, vitamin D, and parathyroid hormone activity, an area where the recent advances in our molecular knowledge have been remarkable. Laurence Chan, an international expert, has written an exciting chapter dealing with the genomic and nongenomic effects of angiotensin and aldosterone in renal and cardiovascular disease as well as updating information on magnesium homeostasis. Diana Jalal, a premier clinician-educator, discusses in a very erudite manner the pivotal role of the kidney in the pathogenesis of hypertensive states. Phyllis August and Line Malha have included the recent advances in understanding the state of preeclampsia and eclampsia in their chapter.

There has been substantial new knowledge regarding the mechanisms of vascular and epithelial kidney injury during ischemia, which is discussed by Charles Edelstein relative to the pathophysiology of acute kidney injury. Kevin Harris discusses the physiology and pathophysiology of urinary tract obstruction. Michel Chonchol and Lawrence Chan have written about the renal advances in chronic kidney disease in understanding and treating this growing problem. Shubha Ananthakrishnan and George A. Kaysen, major contributors to our understanding of proteinuric states, share that pioneering knowledge in the nephrotic syndrome chapter. The advances in our understanding of the glomerulopathies and vasculitides have never been greater and Joshua Thurman has completely and authoritatively written this chapter.

In the over 40 years of *Renal and Electrolyte Disorders*, there has never been an opportunity to present so much new knowledge as this eighth edition. We are very fortunate to have these distinguished authors provide this exciting eighth edition for our readers. I also want to thank Jan Darling for her excellent editorial support.

ROBERT W. SCHRIER

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CHAPTER 1

Disorders of Water Homeostasis

Tomas Berl and Robert W. Schrier

Historical and Evolutionary Aspects of Renal Concentrating and Diluting Processes

In *From Fish to Philosopher*, Smith (1) suggested that the concentrating capacity of the mammalian kidney may have played an important role in the evolution of various biologic species, including Homo sapiens. He suggested that the earliest protovertebrates resided in a saltwater environment that had a composition similar to their own extracellular fluid (ECF); therefore, these species could ingest freely from the surrounding sea without greatly disturbing the composition of their own *milieu interieur*. However, when these early vertebrates migrated into freshwater streams, the evolution of a relatively water-impermeable integument was mandatory to avoid fatal dilution from their hyposmotic, freshwater environment. Thus a vascular tuft—which we now call the glomerulus—developed, enabling the fish to filter the excess fluid from their blood.

The proximal tubule, which reabsorbed isotonic fluid, evolved in response to the need for salt preservation. However, this did not allow the excretion of hypotonic urine, which is critical for the survival of organisms ingesting hypotonic fluid from their freshwater environment. This need was met by the development of the distal tubule, which could dilute

tubular fluid and ultimately urine. This dilution is accomplished by reabsorption of salt without water, because the distal tubular epithelium was relatively impermeable to water. The fish then could excrete the excess solute-free water they had obtained from their freshwater environment while concomitantly conserving their body salts.

Vertebrates began to reside on dry land several million years later. The problem of salt conservation persisted in this terrestrial environment, but the excretion of large volumes of dilute fluid was no longer necessary. Rather, conservation of fluid became of primary importance in the new arid environment. The kidneys of reptiles, birds, and mammals, however, had glomeruli, which filtered large amounts of fluid and salt, even though excretion of only minute amounts of these substances was needed to maintain daily balance. In reptiles and birds, the kidneys responded to this challenge by a decrease in the number of capillary loops in their glomerular tufts. Aglomerular kidneys even evolved in some fish, such as the sea horse and pipefish, which may have been the first vertebrates to return to the sea. Tubular secretory systems evolved in these nephrons to allow elimination of nitrogenous wastes without the need for large volumes of filtered fluid. Also, a relatively insoluble nitrogenous end product, uric acid, was produced that could be excreted in supersaturated solutions with minimal water loss.

The high-pressure glomerular filters were maintained in mammals; however, the countercurrent mechanism developed for concentrating urine. Mammals, along with birds, are unique among vertebrates in possessing loops of Henle and in their ability to compensate for water deficits by elaborating urine more concentrated than blood.

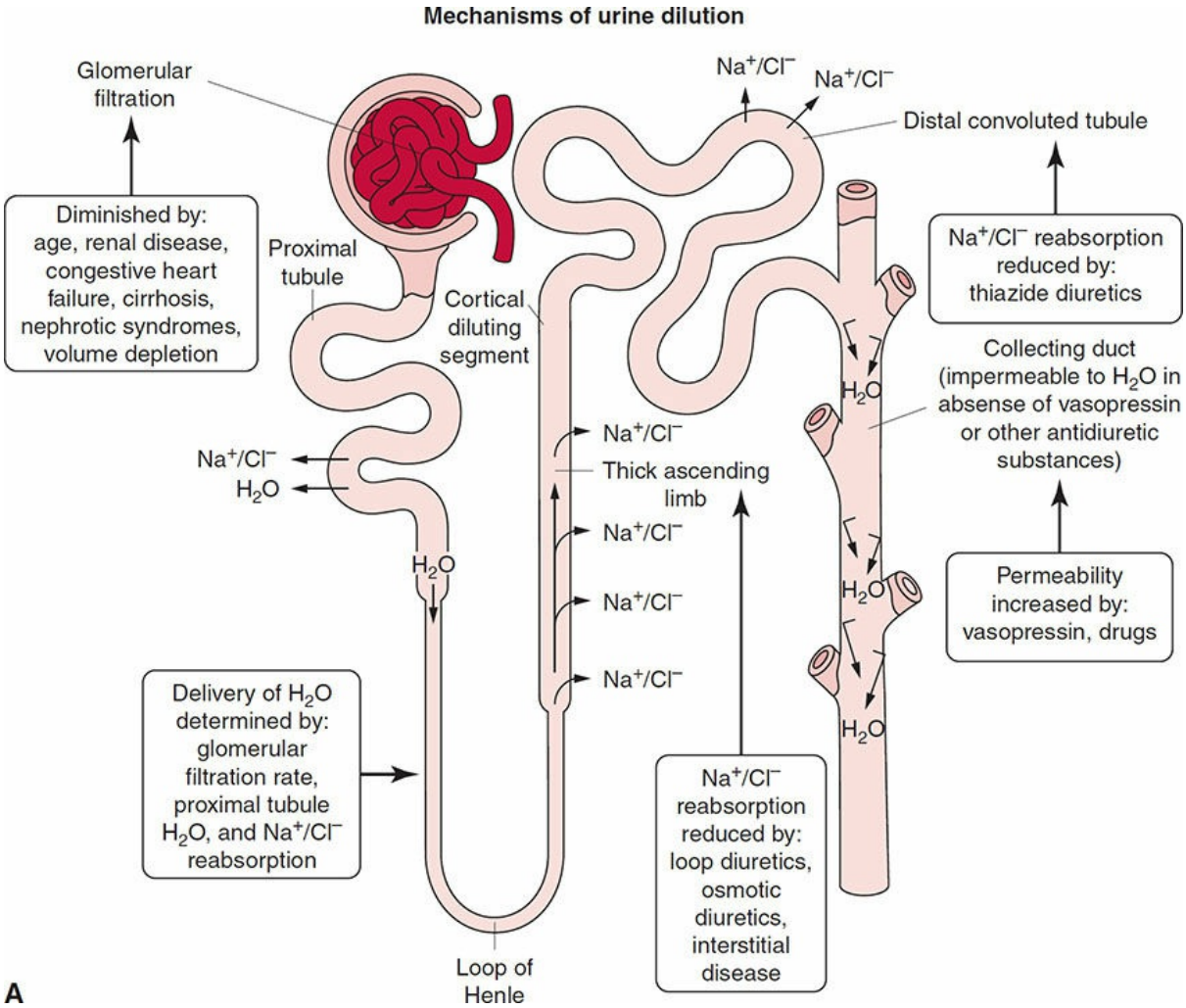
Countercurrent Concentrating Mechanism

By analogy with heat exchangers, the functional significance of the loops of Henle was proposed when Kuhn and Ryffel of the physical chemistry department at the University of Basel, Switzerland, originated the concept of the countercurrent multiplier system for urine concentration in 1942 (2). The hypothesis states that a small difference in osmotic concentration (single effect, or *einzeln Effekt*) at any point between fluid flowing in opposite directions in two parallel tubes connected in hairpin manner can be multiplied many times along the length of the tubes. In the kidney, a small, 200 mOsm gradient results in a large osmolar concentration difference between the corticomedullary junction and the hairpin loop at

the tip of the papilla. Since then, although numerous experiments have confirmed the overall operation of a countercurrent multiplier in the kidney, with the thick ascending limb of Henle as the water-impermeable site of active solute reabsorption (3), the precise mechanism that culminates in the generation of the medullary osmotic gradient is not fully understood (4).

URINARY CONCENTRATION AND DILUTION

As disturbances in the capacity of the kidney to concentrate and dilute the urine are central to the pathogenesis of disorders of water balance, we will briefly review the components of the diluting and concentrating process in the mammalian kidney. These are depicted in Figure 1-1A and B, respectively (5). It is important to emphasize that many of these processes are the same whether the final excreted urine is hypotonic or hypertonic to plasma.



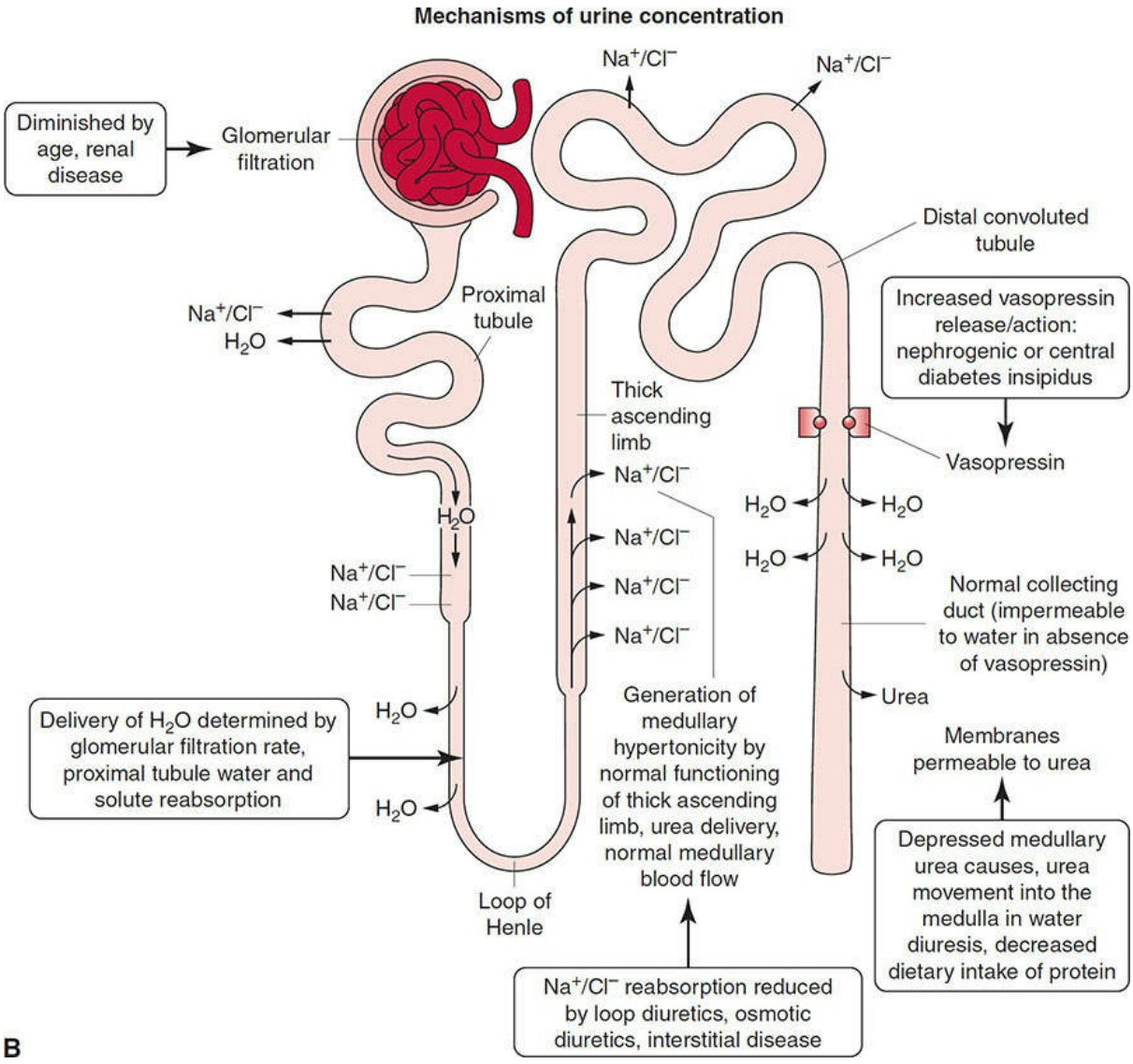


Figure 1–1 (**A**) Urinary dilution mechanisms. Normal determinants of urinary dilution and disorders causing hyponatremia. (**B**) Urinary concentrating mechanisms. Determinants of normal urinary concentrating mechanism and disorders causing hypernatremia. (From Berl T, Parikh C. Disorders of water metabolism. In: London MI, ed. *Comprehensive Clinical Nephrology*. 5th ed. Philadelphia: Saunders; 2014:94–110.)

Glomerular Filtration Rate and Proximal Tubular Reabsorption

The rates of glomerular filtration and proximal tubular reabsorption are important primarily in determining the rate of sodium and water delivery to the more distal portions of the nephron, where the renal concentrating and diluting mechanisms are operative. Fluid reabsorption in the proximal tubule is isosmotic; therefore, tubular fluid is neither concentrated nor diluted in the proximal portion of the nephron. Rather, after approximately

70% of glomerular filtrate is reabsorbed in the proximal tubules, the remaining 30% of fluid entering the loop of Henle is still isotonic to plasma. The reabsorption of sodium chloride is primarily driven by the Na/H 3 transporter whereas the isotonic removal of water is facilitated by the robust expression of the water channel aquaporin 1 (AQP_1), depicted in Figure 1-2. A decrease in glomerular filtration rate (GFR) or an increase in proximal tubular reabsorption, or both, may diminish the amount of fluid delivered to the distal nephron and thus limit the renal capacity to excrete water. Similarly, a diminished GFR and increased proximal tubular reabsorption may limit the delivery of sodium chloride to the ascending limb, where the tubular transport of these ions without water initiates the formation of the hypertonic medullary interstitium. With diminished delivery of sodium chloride to the ascending limb, the resultant lowering of medullary hypertonicity impairs maximal renal concentrating capacity.

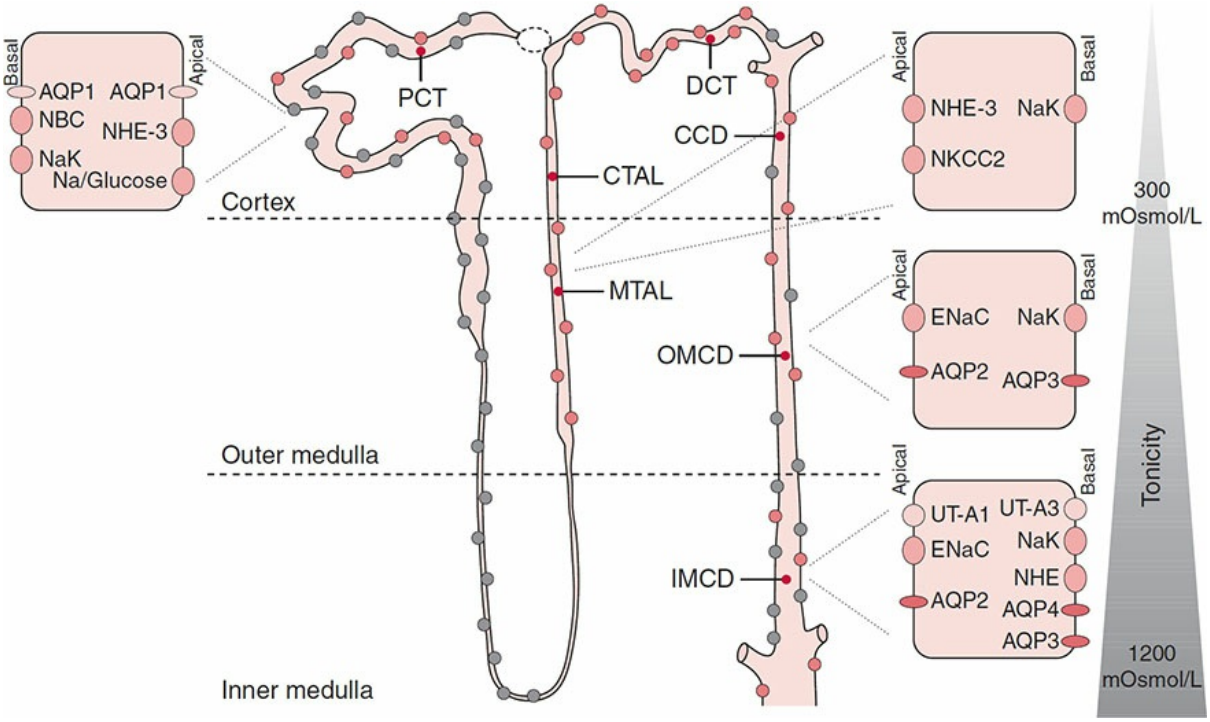


Figure 1–2 Schematic representation of key elements of the kidney tubule that mediate water reabsorption. Direct mediators are those involved in sodium (depicted as *red circles*), urea (*green circles*), and water (*blue circles*) transport. PCT, proximal convoluted tubule; CTAL, cortical thick ascending limb; MTAL, medullary thick ascending limb; DCT, distal convoluted tubule; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct; AQP, aquaporin; NHE, Na^+/H^+ exchanger; Na/Glucose, sodium–glucose cotransporter; NBC, sodium bicarbonate cotransporter; NaK, Na^+/K^+ ATPase; NKCC2, $Na^+/2Cl^-/K^+$ cotransporter; ENaC, epithelial sodium channel; UT-A, urea transporter A isoform. (From Hasler U, Leroy V, Martin PY, et al. Aquaporin-2 abundance in the renal

collecting duct: new insights from cultured cell models. *Am J Physiol Renal Physiol*. 2009;(297:1), with permission.)

Descending and Ascending Limbs of the Loops of Henle, Distal Tubule, and Collecting Ducts

Because the urine that emerges from the proximal tubule is isosmotic, the first nephron segment actually involved in urinary concentration is the descending limb of Henle's loop. There are two types of descending limbs. The short loops originate in superficial and mid cortical glomeruli and turn in the outer medulla. The long loops originate in deep cortical and juxtamedullary glomeruli and penetrate variable distances into the inner medulla. Short and long descending limbs are anatomically distinct; the long limbs in particular display considerable interspecies variability (6). Interestingly, no correlation is apparent between a species' maximal concentrating ability and the ratio of short and long loops. In fact, in rodents with highest urinary concentrations, the number of short loops is considerably greater than the number of long loops. Approximately 15% of nephrons possess long loops in the human kidney; the other 85% of nephrons have short loops. The descending thin limb is very water permeable as it also has, in its first portion, abundant expression of AQP_1 (7). Thus, tubular fluid is concentrated as it descends primarily, but probably not exclusively, by the extraction of water.

Somewhat proximal to the hairpin turn, there is a transition from the descending limb to the ascending thin limb of Henle's loop. This segment, as well as the remainder of ascending limb, is water impermeable, As will be further discussed, the nature and particular site at which the movement of solutes (urea and NaCl) occur has not been fully defined. Active sodium transport has not been demonstrated convincingly, and this segment's morphologic appearance with few mitochondria does not suggest active metabolic work.

The thick ascending limb of Henle's loop appears both structurally and functionally distinct from its thin counterpart. The epithelium is remarkably uniform among species with tall, heavily interdigitating cells with large mitochondria. The observation that fluid emerges into the early distal tubule hypotonic (about 100 mOsm/kg H_2O) supports the view that active sodium chloride transport out of this water-impermeable segment provides the single effect required for the operation of the countercurrent multiplier. The primary mechanism of chloride absorption in the thick ascending limb is mediated by an electroneutral sodium, potassium, and

chloride (Na^+:K^+:$2Cl^-$) cotransport (Fig. 1-2).

The distal convoluted tubule is the segment between the macula densa and collecting ducts. This is a morphologically heterogenous segment (6) that is also water impermeable and unresponsive to vasopressin. The collecting ducts are formed in the cortex by the confluence of several distal tubules. They descend through the cortex and outer medulla individually, but successively fuse together on entering the inner medulla. In humans, a terminal inner medullary collecting duct draws from as many as 7,800 nephrons. The collecting ducts possess vasopressin-sensitive adenylate cyclase in all species studied; they are virtually impermeable to water in the absence of the hormone. The vasopressin-sensitive water channel AQP_2 mediates water reabsorption in this segment of the nephron in concert with AQP_3 and AQP_4 (7,8). The collecting duct in its cortical and medullary segments is also impermeable to urea, but in response to the vasopressin-sensitive urea transporter UT 1, the inner medullary collecting duct is rendered urea permeable (9).

Kokko and Rector, as reviewed by Sands (10), have proposed a model of urinary concentration that is in concert with the anatomic features and the permeability characteristics of the various segments of the system, while limiting the active transport of solute to the thick portion of the ascending limb of Henle in the outer medulla. The components of the mechanism, as depicted in Figure 1-3A, are as follows:

1. The water-impermeable thick ascending limb of Henle's loop actively cotransports sodium, chloride, and potassium, thereby increasing the tonicity of the surrounding interstitium and delivering hypotonic fluid to the distal tubule. Urea is poorly reabsorbed and therefore retained in the tubule.
2. Under the influence of vasopressin in the cortical and outer medullary collecting ducts, tubular fluid equilibrates with the isotonic and hypertonic interstitium, respectively. Low urea permeability in this portion of the nephron allows its concentration to further increase.
3. In the presence of vasopressin, the inner medullary collecting duct is rendered more permeable to urea. Therefore, in this segment of nephron, in addition to water reabsorption, urea is reabsorbed as it diffuses passively along its concentration gradient into the interstitium, where it constitutes a significant component of the medullary interstitial tonicity.
4. The resulting increase in interstitial tonicity creates the osmotic gradient that abstracts water from a highly water permeable and solute

impermeable descending limb of Henle's loop. This process elevates the concentration of sodium chloride in the tubular fluid. When tubular fluid arrives at the bend of the loop, its tonicity is the same as that of the surrounding interstitium. However, the sodium chloride concentration of the tubular fluid is higher and the urea concentration lower than that of the interstitium.

5. Tubular fluid then enters the thin ascending limb, which is more permeable to sodium than urea. The sodium gradient provides for passive removal of sodium chloride from this segment into the interstitium.

To prevent urea removal from the inner medulla to the cortex, the ascending and descending vasa recta act as a countercurrent exchanger and "trap" urea in the inner medulla. The ascending vasa recta also may deposit urea into adjacent descending thin limbs of a short loop of Henle, thereby recycling it to the inner medullary collecting tubule. The descending limbs of short loops do not enter the inner medulla; thus, the addition of urea to these loops does not interfere with the removal of water from the descending thin limb in the inner medulla, a step that is so crucial to the concentrating process.

This passive model of urinary concentration has a number of attractive features, and many of its aspects have been experimentally supported (10). However the requisite difference in sodium and urea permeabilities that is needed for this model to operate passively (point 5 above) have not been met, as mathematical models that employ available permeabilities fail to generate the desired osmotic gradients. Thus, alternatives have been tested. Among these, a three-dimensional reconstruction of the components of the rat inner medulla coupled with the development of mathematical models has emerged with a variation of the previous model, designated as the solute mixing passive model, depicted in Figure 1-3B (4). This model recognizes that the water permeability of the descending limb does not extend into the inner medulla as AQP_1 is absent in the lower half of this limb of Henle's loop. The high urea permeability and the passive exit of sodium from tubular fluid occur before the bend of the loop, equally in the descending as well as ascending thin loops rather than exclusively in the latter. Although this model predicts the generation of concentrated urine, it does not produce one that is maximally concentrated (4).

What remains widely agreed upon is that the single effect in the ascending limb of Henle, so critical to the operation of the countercurrent system and urinary concentration, also serves to dilute the urine. In the

absence of vasopressin, and thus with water impermeability of the collecting ducts, the continued reabsorption of solute in the remainder of the distal nephron results in a maximally dilute urine (50 mOsm/kg). Thus, it should be apparent that impairment of sodium, chloride, and potassium cotransport in the ascending limb of the loop of Henle will limit the renal capacity both to concentrate and to dilute the urine.

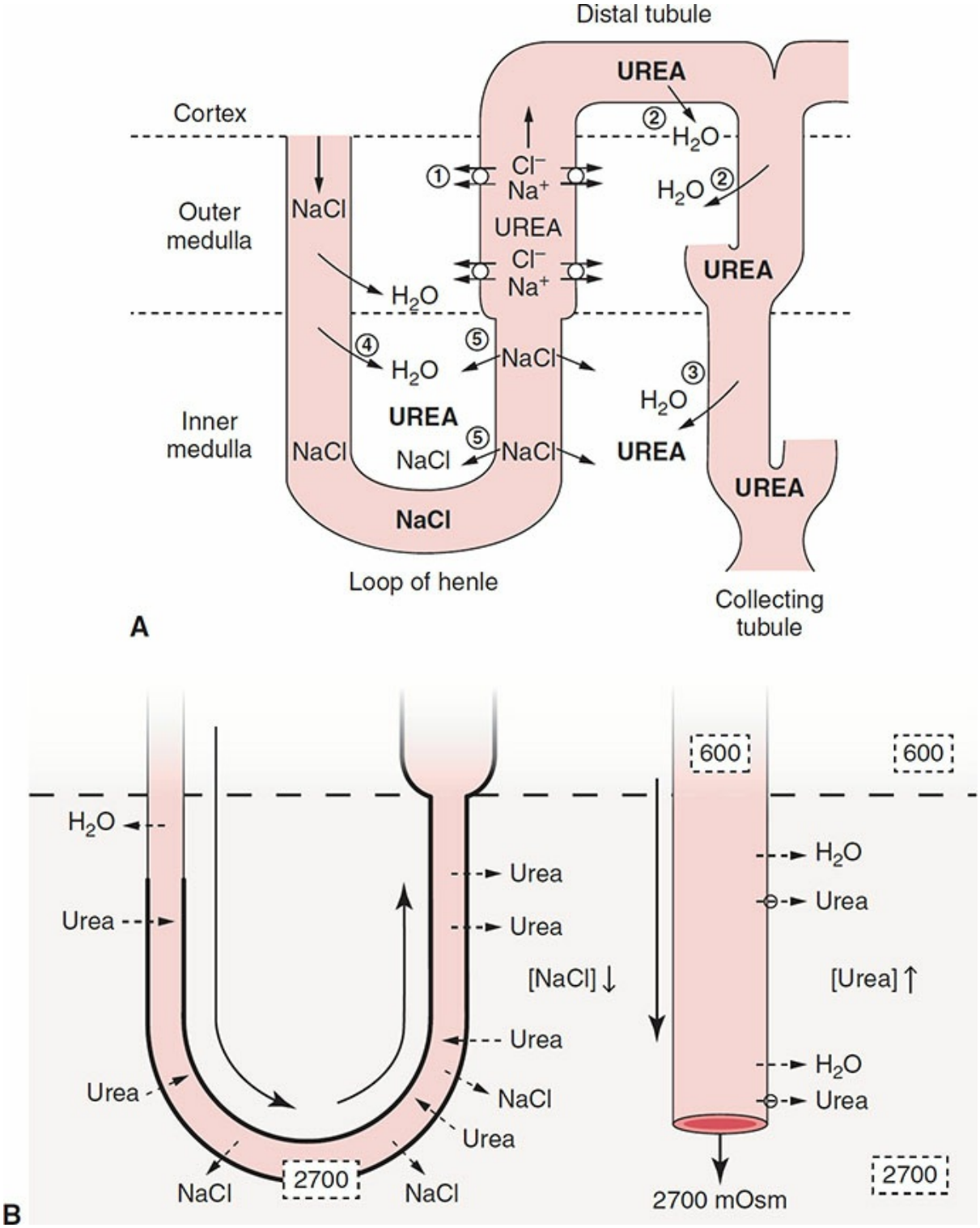


Figure 1–3 (**A**) Schematic representation of the passive urinary concentrating mechanism. Both the thin ascending limb in the inner medulla and the thick ascending limb in the outer medulla, as well as the first part of the distal tubule, are impermeable to water, as indicated by the thickened lining. In the thick ascending limb, active sodium, chloride, and potassium cotransport renders the tubule fluid dilute and the outer medullary interstitium hyperosmotic (*1*). In the last part of the distal tubule and the collecting tubule in the cortex and outer medulla, water is reabsorbed down its osmotic gradient (*2*), increasing the concentration of urea that remains behind. In the inner medulla, both water and urea are reabsorbed from the collecting duct (*3*). Some urea reenters the loop of Henle (data not shown). This medullary recycling of urea, in addition to trapping of urea by countercurrent exchange in the vasa recta (data not shown), causes urea to accumulate in large quantities in the medullary interstitium (indicated by UREA), where it osmotically extracts water from the descending limb (*4*) and thereby concentrates sodium chloride in descending limb fluid. When the fluid rich in sodium chloride enters the sodium chloride-permeable (but water-impermeable) thin ascending limb, sodium chloride moves passively down its concentration gradient (*5*), rendering the tubule fluid relatively hyposmotic to the surrounding interstitium. (From Jamison RL, Maffly RH. The urinary concentrating mechanism. *N Engl J Med*. 1976;295(19):1059, Copyright ¿ 2017 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.) (**B**) Illustrating the unique permeabilities and solute fluxes of current solute separation, solute mixing passive model for concentrating urine. Thick tubule border indicates AQP_1-null, water-impermeable segment of DTL as well as water-impermeable ATL and TAL. The AQP_1-null segment of the DTL is essentially impermeable to inorganic solutes and water. In this model, both the ATLs and the DTLs (including the AQP_1-null segment) are highly permeable to urea. In contrast to the original passive model, passive NaCl reabsorption without water begins with the prebend segment and is most significant around the loop bend. Also, in contrast to previous models, urea moves passively into the entire DTL and early ATL, but as this urea-rich fluid further ascends in the ATL, it reaches regions of lower interstitial urea concentration and diffuses out of the ATL again. Thus, the loops act as countercurrent exchangers for urea. (Republished with permission of American Society of Nephrology, from Dantzler W. et al Urine-concentrating mechanism in the inner medulla: function of the thin limbs of the loops of Henle. *Clin J Am Soc Nephrol*. 2014;9(10):1781–1789; permission conveyed through Copyright Clearance Center, Inc.)

Medullary Blood Flow

Medullary blood flow, whose rate can be regulated independently of whole kidney blood flow, may also affect the renal capacity both to concentrate and to dilute the urine, because the preservation of the medullary hypertonicity in the interstitium is dependent on the countercurrent exchange mechanism in the vasa recta. Although medullary blood flow constitutes only 5% to 10% of total renal blood flow, this flow is still

several times more rapid than the tubular flow. The vasa recta possess AQP_1 and serve as a countercurrent exchanger that permits the preservation of interstitial tonicity. Blood that enters the descending vasa recta becomes increasingly concentrated as water diffuses out of and solutes diffuse into this portion of the nephron. The hairpin configuration of the vasa recta, however, does not allow the solute-rich blood to leave the medulla. In the ascending portion of the vasa recta, water diffuses into the vasa recta and solute moves out, thus maintaining interstitial hypertonicity. Even with an intact countercurrent exchange system in the vasa recta, circumstances that increase medullary blood flow may "wash out" the medullary concentration gradient and thereby diminish renal concentrating capacity. Moreover, even in the absence of vasopressin, the collecting duct is not completely water impermeable; therefore, a further decrease in the hypertonic medullary interstitium during an increase in medullary blood flow may decrease the vasopressin-independent osmotic water movement from the collecting duct and thereby increase water excretion.

Distal Solute Load

The rate of solute delivery to the collecting duct is a known determinant of renal concentrating capacity. As depicted in Figure 1-4, in spite of maximal levels of vasopressin, urinary osmolality in normal humans progressively diminishes as solute excretion increases. At high rates of solute excretion, urinary osmolality may reach isotonicity in humans, even though supraphysiologic doses of vasopressin are infused. An increase in solute excretion even may be associated with hypotonic urine with the infusion of submaximal doses of vasopressin in patients with pituitary diabetes insipidus. At least two factors may be responsible for this effect of solute excretion on renal concentrating capacity. First, a solute diuresis generally is associated with an increase in medullary blood flow, which could lower the medullary solute concentration profile. Second, the rapid rate of tubular flow through the medullary collecting duct could shorten contact time sufficiently so that complete osmotic equilibrium of fluid would not be allowed between the collecting duct and medullary interstitium, even though vasopressin had made the collecting duct membrane maximally permeable to water.

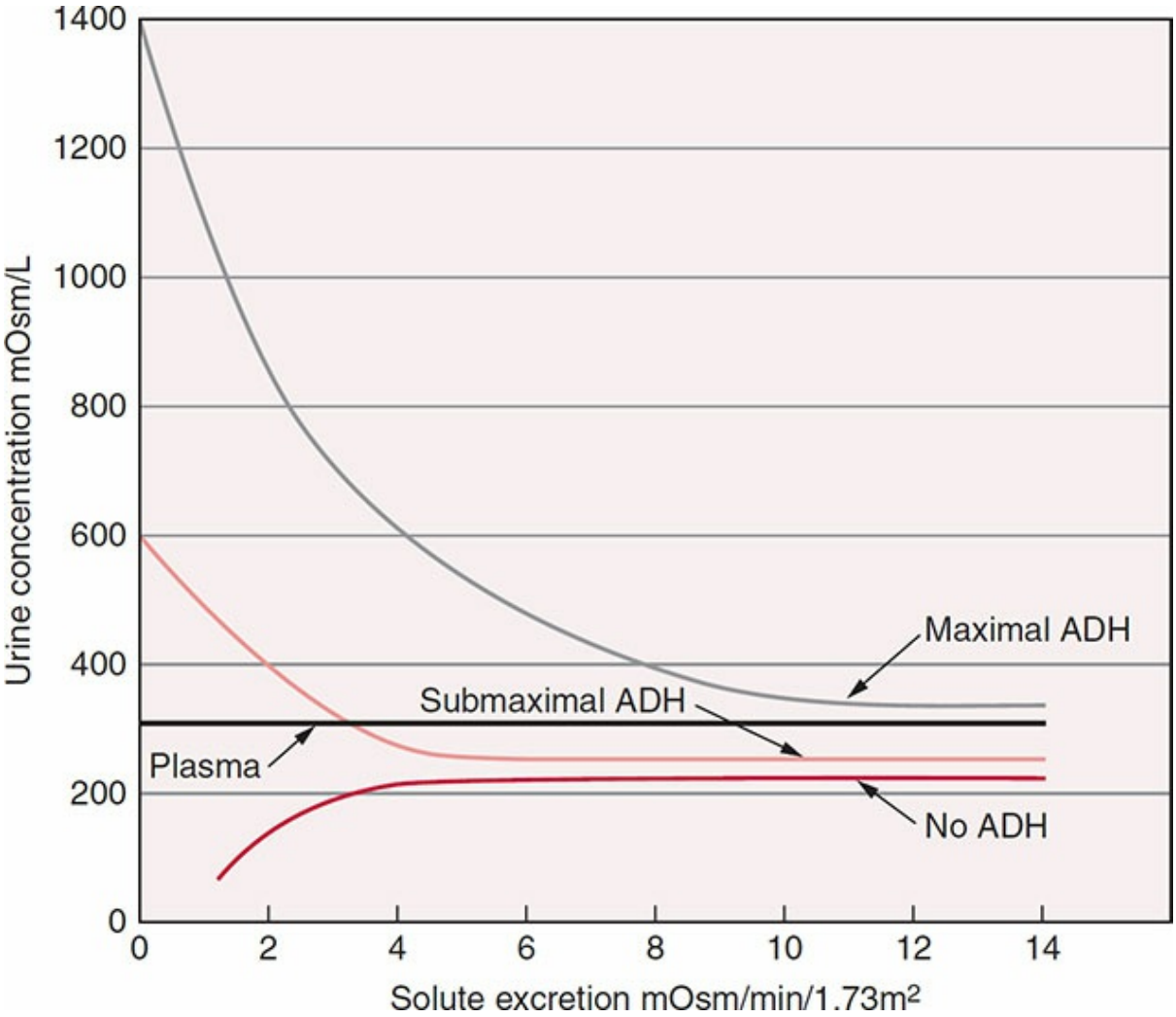


Figure 1–4 Effect of solute excretion on renal concentration and diluting mechanisms. The submaximal response to antidiuretic hormone (ADH) may result from the presence of submaximal amounts of ADH or the diminished response of the collecting duct to maximal amounts of ADH. (Reproduced from de Wardener HE, del Greco F. Influence of solute excretion rate on production of hypotonic urine in man. *Clin Sci*. 14(4):715–723. © 1955, the Biochemical Society.)

Antidiuretic Hormone

The renal concentrating and diluting processes are ultimately, and most importantly, dependent on the presence or absence respectively of arginine vasopressin (AVP) to modulate the water permeability of the collecting duct. AVP, a cyclic hexapeptide (mol wt 1,099) with a tail of three amino acids, is the antidiuretic hormone (ADH) in humans (Fig. 1-5). The presence of a basic amino acid (arginine or lysine) in the middle of the intact hormone at position 8 is crucial for antidiuresis, as is the asparagine at position 5. AVP is synthesized in the supraoptic and paraventricular magnocellular nuclei in the hypothalamus. In these nuclei, a biologically

inactive macromolecule is cleaved into the smaller, biologically active AVP. Both oxytocin and AVP are encoded in human chromosome 20 in close proximity to each other, depicted in Figure 1-6. The preprohormone gene is approximately 2,000 base pairs in length and comprises three exons (Fig. 1-6). AVP is encoded in the first exon following a signal peptide. Although spanning all three exons, the binding protein neurophysin is encoded primarily in exon B and the terminal glycoprotein in exon C. The promoter has *cis*-acting elements, including a glucocorticoid response element, a cyclic adenosine monophosphate (cAMP) response element, and four AP-2 binding sites (11). The precursor prohormone, called propressophysin, is cleaved by removal of the signal peptide after translation. Vasopressin, with its binding protein neurophysin II, and the glycoprotein are transported in neurosecretory granules down the axons and stored in nerve terminals in the pars nervosa. There is no known physiologic role of the neurophysins, but they neutralize the negative charge of vasopressin. The release of stored peptide hormone and its neurophysin into the systemic or hypophyseal portal circulation occurs by an exocytosis. With increased plasma osmolality, electrical impulses travel along the axons and depolarize the membrane of the terminal axonal bulbs. The membrane of the secretory granules fuses with the plasma membrane of the axonal bulbs, and the peptide contents are then extruded into the adjacent capillaries. The Brattleboro rat, a strain with an autosomal recessive defect that causes AVP deficiency, is afflicted by a single base deletion in exon B. This leads to a shift in the reading frame, with loss of the translational stop code. Although transcribed and translated in the hypothalamus, the translational product is neither transported nor processed in these mutant rats.

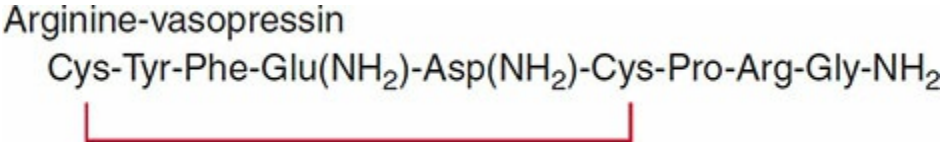


Figure 1–5 Structure of the human antidiuretic hormone, arginine vasopressin. (From Schrier RW, Miller PD. Water metabolism in diabetes insipidus and the syndrome of inappropriate antidiuretic hormone secretion. In: Kurtzman NA, Martinez Maldonado M, eds. *Pathophysiology of the Kidney*. Springfield, IL: Charles C Thomas; 1977.)

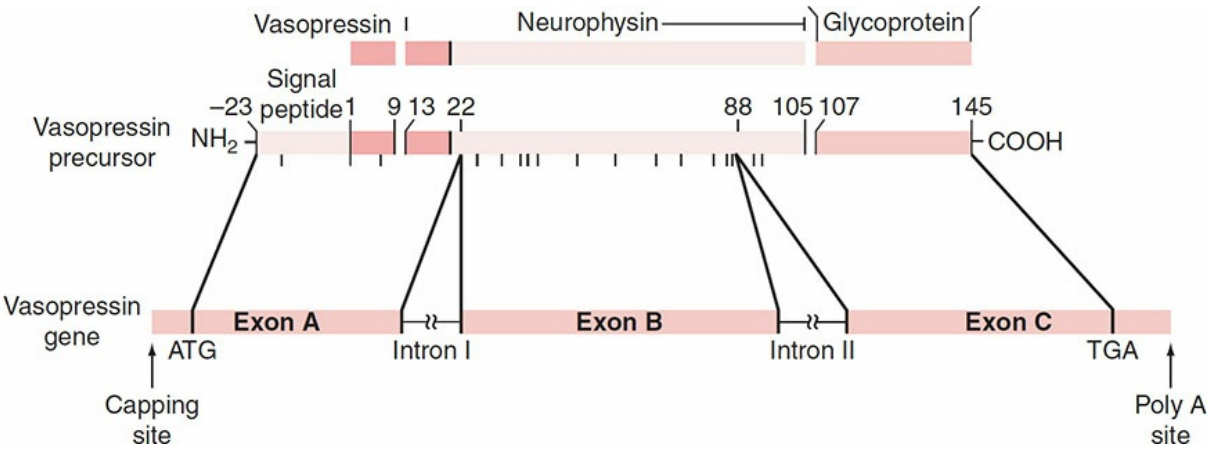


Figure 1–6 The arginine vasopressin (AVP) gene and its protein products. The three exons encode a 145-amino acid prohormone with an NH-2-terminal signal peptide. The prohormone is packaged into neurosecretory granules of magnocellular neurons. During axonal transport of the granules from the hypothalamus to the posterior pituitary, enzymatic cleavage of the prohormone generates the final products: AVP, neurophysin, and a COOH-terminal glycoprotein. When afferent stimulation depolarizes the AVP-containing neurons, the three products are released into capillaries of the posterior pituitary. (From Brenner BM, ed. *The Kidney*. 8th ed. Philadelphia: Saunders Elsevier; 2008, with permission.)

The regulation of AVP release from the posterior pituitary is dependent primarily on two mechanisms: osmotic and nonosmotic pathways (Fig. 1-7).

Osmotic Release of Vasopressin

The osmotic regulation of AVP is dependent on “osmoreceptor” cells in the anterior hypothalamus in proximity but separate from supraoptic nuclei. These cells, most likely by altering their cell volume, recognize changes in ECF osmolality. Cell volume is decreased most readily by substances that are restricted to the ECF, such as hypertonic saline or hypertonic mannitol, and thus enhance osmotic water movement from cells; these substances are very effective in stimulating AVP release. Since the effects of saline and mannitol are comparable, this supports the view that the response is due to changes in effective osmolality rather than to sodium per se. In contrast, urea moves readily into cells and therefore does not alter cell volume; hypertonic urea does not effectively stimulate AVP release. The effects of increased osmolality on vasopressin release are associated with measurable (twofold to fivefold) increases in vasopressin precursor messenger RNA (mRNA) in the hypothalamus. The osmoreceptor cells are very sensitive to changes in ECF osmolality. An

increase of ECF osmolality by 1% stimulates AVP release, whereas water ingestion causing a 1% decrease in ECF osmolality suppresses AVP release (Fig. 1-8). A role for members of the transient receptor potential vallinoid family (TRPV 1 and 4) in osmoregulation has been suggested as knockouts of these proteins in mice become somewhat hypernatremic and display blunted vasopressin secretion in response to hypertonic stimuli as summarized by Cohen (12).

A close correlation between AVP and plasma osmolality has been demonstrated in subjects with various states of hydration, although there are considerable genetically determined individual variations in both the threshold and sensitivity (Fig 1-8). In humans, the osmotic threshold for vasopressin release is between 280 and 290 mOsm/kg. The system is so efficient that plasma osmolality usually does not vary more than 1% to 2 %, despite great variations in water intake. There is also a close correlation between AVP and urinary osmolality, allowing for the maintenance of tonicity of body fluids.

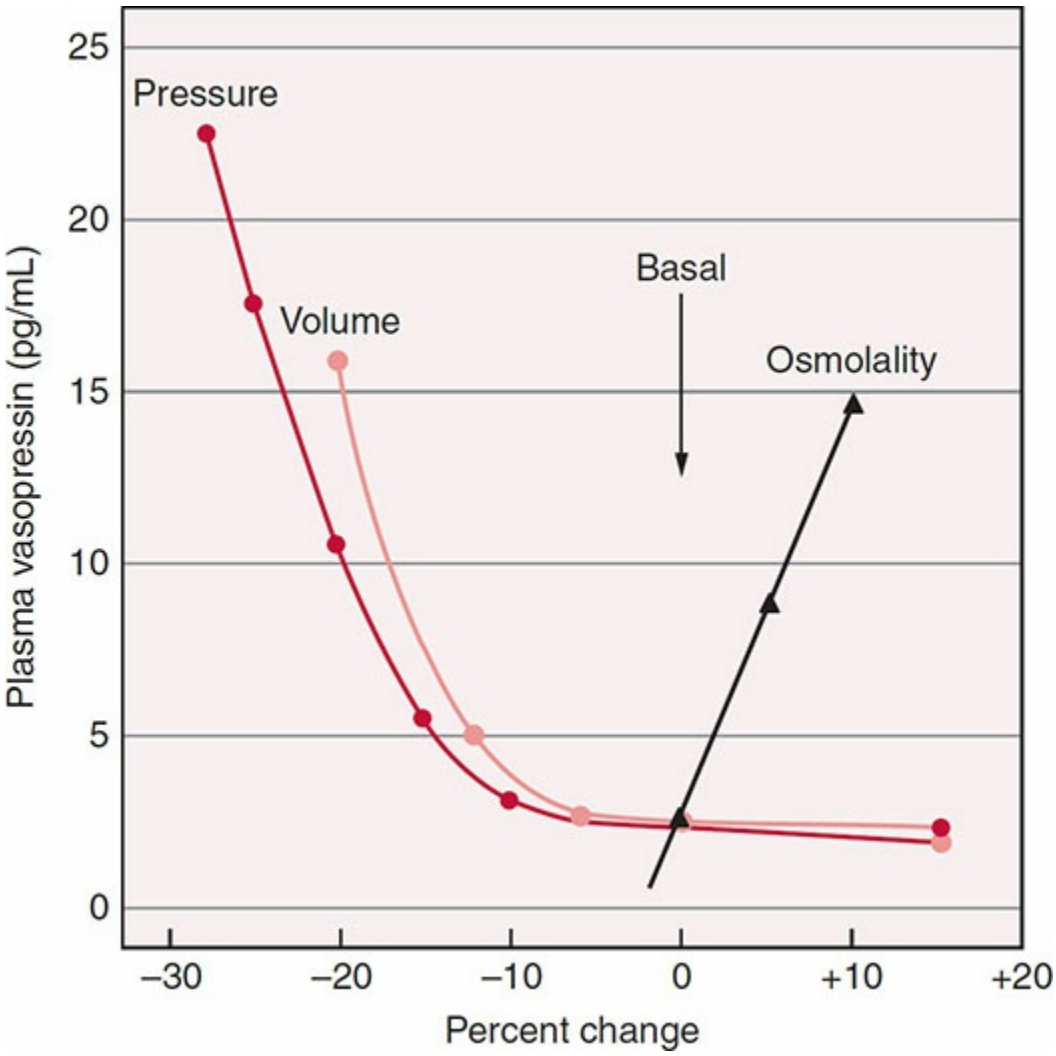


Figure 1–7 Osmotic and nonosmotic stimulation of arginine vasopressin release. (From Robertson GL, Berl T. Pathophysiology of water metabolism. In: Brenner BM, ed. *The Kidney*. 6th ed. Philadelphia: WB Saunders; 2000:875, with permission.)

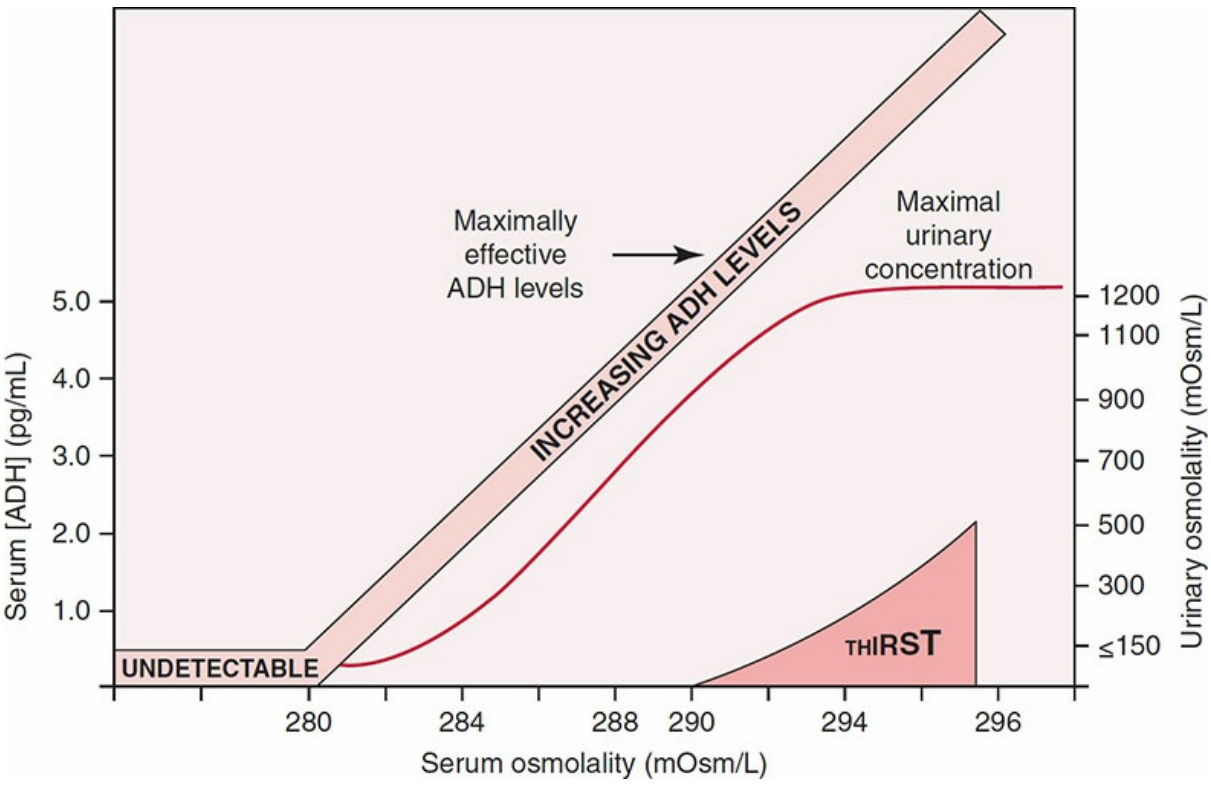


Figure 1–8 Antidiuretic hormone levels, urinary osmolality, and thirst as functions of serum osmolality. (From Narins RG, Krishna GC. Disorders of water balance. In: Stein JH, ed. *Internal Medicine*. Boston: Little, Brown; 1987:794, with permission.)

Nonosmotic Release of Vasopressin

Vasopressin release can occur in the absence of changes in plasma osmolality (5). Although a number of such nonosmotic stimuli exist, physical pain, emotional stress, and a decrement in blood pressure or volume are the most prominent ones. A 7% to 10% decrement in either blood pressure or blood volume causes the prompt release of vasopressin (Fig. 1-7). Because the integrity of the circulatory volume takes precedence over mechanisms that maintain tonicity, activation of these nonosmotic pathways overrides any decline in the osmotic stimulus that otherwise would suppress the hormone's release. This process accounts for the pathogenesis of hyponatremia in various pathophysiologic states, including cirrhosis, heart failure, and several endocrine disorders.

There is considerable evidence for the existence of baroreceptor sensors in the low-pressure (venous) areas of the circulation, particularly in the atria. Atrial distention causes a decrease in plasma AVP levels, and

a water dieresis; this reflex is mediated by the vagus nerve. Alternatively, arterial baroreceptors in the aorta and carotid sensors send impulses through the vagus and glossopharyngeal nerves to the nucleus tractus solitarii of the medulla. Unloading of these arterial baroreceptors decreases tonic inhibition and leads to the nonosmotic release of vasopressin. Denervation of these arterial baroreceptors has been shown to abolish the nonosmotic release of AVP.

It is possible that angiotensin II is a mediator of AVP release in these states because many of the pathophysiologic states associated with nonosmotic AVP release are characterized by enhanced plasma renin activity and therefore increased angiotensin II levels. The experimental results in this regard are however conflicting. Activation of the sympathetic nervous system seemed be involved in the nonosmotic stimulation of AVP. In this regard, the supraoptic nuclei are heavily innervated by noradrenergic neurons. Other pathways that could stimulate the nonosmotic secretion of AVP have been proposed; for example, the antidiuresis associated with nausea and pain has been ascribed to an emetic and to a cerebral pain center, respectively. A role for baroreceptor pathways however has not been convincingly excluded even in these settings. Other biogenic amines, polypeptides, and even cytokines have been implicated as modulators of AVP release in addition to catecholamines.

Cellular Action of Vasopressin

Once released from the posterior pituitary, vasopressin exerts its biologic action on water excretion by binding to V_2 receptors in the basolateral membrane of the collecting duct (Fig. 1-9) (13). The receptors to which vasopressin binds have been cloned. The V_1 receptor on blood vessels and elsewhere is a 394 amino acid protein with seven transmembrane domains (14). The 370 amino acid V_2 receptor, which is present only in the kidney and has a similar configuration, has been cloned for both rats (15) and humans (16). Although the V_1 receptor messenger is plentiful in the glomerulus, it is also detected in the collecting duct, where the V_2 message is predominant.

Binding of AVP to its V_2 receptor increases adenylate cyclase activity resulting in the generation of cyclic adenosine 3′,5′-monophosphate (cAMP) from adenosine triphosphate. The V_2 receptor is coupled to the catalytic unit of adenylate cyclase by the stimulatory guanine nucleotide

binding regulatory protein, Gs. This is a heterotrimeric protein whose α subunit binds and hydrolyzes guanosine triphosphate. The heightened cAMP formation activates protein kinase A (PKA), which in turn phosphorylates serines and threonines. The activation of PKA brings about the phosphorylation of the water channel AQP_2 at serine 256 in intracellular vesicles, and thereby increases the trafficking of the water channel to the luminal membrane (17). This sequence of events results in a marked increase in the water permeability of the luminal membrane and thereby the collecting tubule. AQP_2 is a member of an increasingly large family of water channels whose archetypal member, AQP_1, cloned by Agre and coworkers (18) is, as mentioned previously, abundant in the proximal tubule and the proximal half of the descending limb of Henle. In contrast, AQP_2 is limited to the vasopressin-sensitive principal cell of the collecting duct, and particularly to the cytoplasm and luminal membrane. Vasopressin also is involved in the long-term regulation of the expression of AQP_2 (19). AQP_3 and AQP_4 are widely distributed, including the collecting duct principal cell, where they are localized at the basolateral membrane. In this basolateral membrane, they serve as conduits for water exit from the cell. Other AQP_6 to AQP_8 also are expressed in the kidney. AQP_6 is present in intercalated cells, AQP_7 in the S_3 segment of the proximal tubules, AQP_8 in proximal tubules and collecting ducts, and AQP_{11} in the proximal tubule (19). The physiologic roles of these water channels in the kidney are not clear. The cytoskeleton also plays an important role in the trafficking of the AQP_2 water channel to the luminal membrane, a process involving both exocytic insertion associated with AVP stimulation and endocytic retrieval associated with suppression of AVP action.

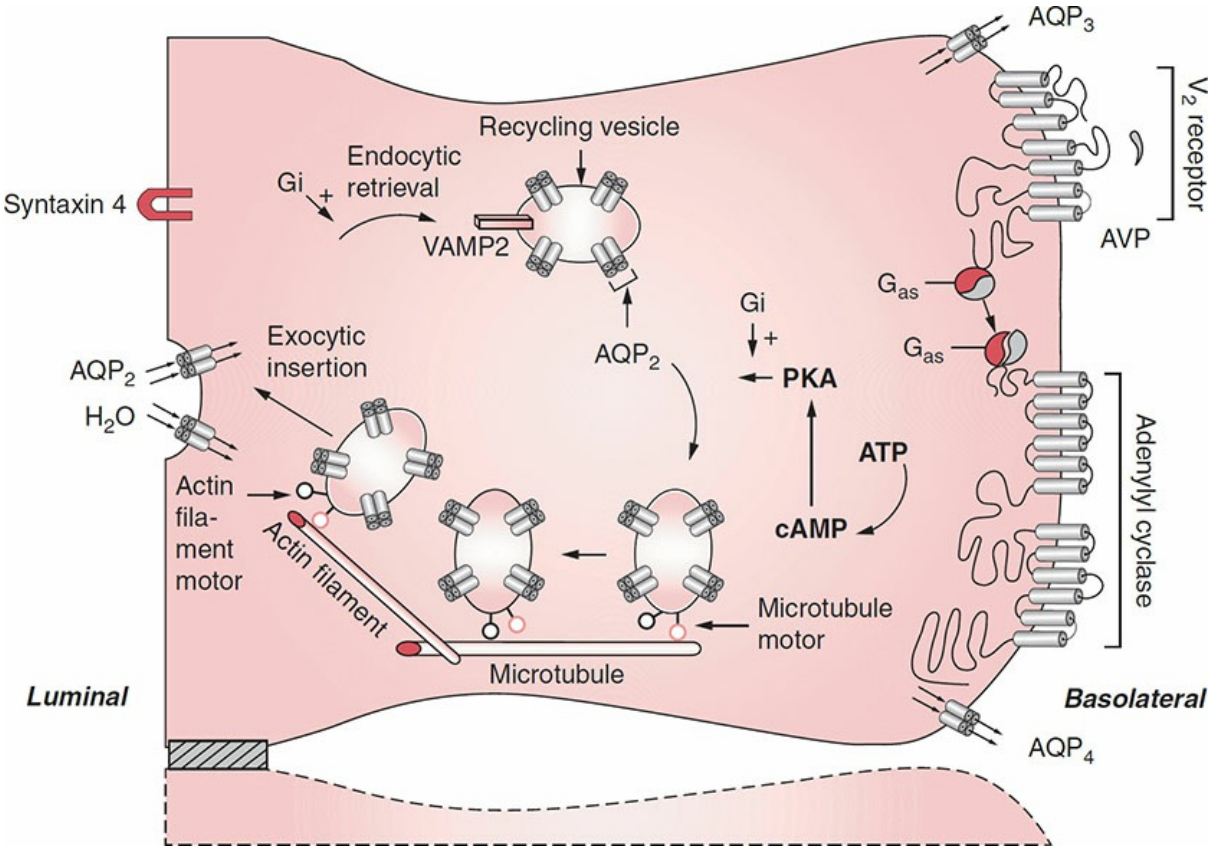


Figure 1–9 Schematic representation of the cellular action of vasopressin. The binding of vasopressin to the V_2 receptor in the basolateral membrane initiates a cascade of events resulting in AQP_2 insertion in the luminal membrane, see text for details. (From Bichet D. Nephrogenic and central diabetes insipidus. In: Schrier RW, ed. *Diseases of the Kidney and Urinary Tract*. Vol 3. 7th ed. New York: Lippincott Williams & Wilkins; 2012:2553, with permission from Wolters Kluwer Health.)

Quantitation of Renal Water Excretion

The quantitation of water excretion has been facilitated by the concept that urine flow (V) is divisible into two components. One component is the urine volume needed to excrete solutes at the concentration of solutes in plasma. This isotonic component has been termed osmolar clearance (C_{osm}). The other component is called solute-free water clearance (C_{H2O}) and is the theoretic volume of solute-free water that has been added to (positive C_{H2O}) or reabsorbed from (negative C_{H2O} or T^c_{H2O}) the isotonic portion of urine (C_{osm}) to create with hypotonic or hypertonic urine, respectively. These terms are calculated as follows:

$$V = C_{osm} + C_{H_2O}$$

$$C_{H_2O} = V - C_{osm}$$

$$\text{Because, } C_{osm} = \frac{\text{Urine osmolality } (U_{osm}) \times \text{urine flow } (V)}{\text{plasma osmolality } (P_{osm})} \quad (1.1)$$

$$C_{H_2O} = V - \frac{U_{osm} + V}{P_{osm}}$$

$$C_{H_2O} = V\left(1 - \frac{U_{osm}}{P_{osm}}\right)$$

Further inspection of these relationships will reveal the following:

1. When U_{osm} equals P_{osm} (isotonic urine), V equals C_{osm}; therefore, C_{H2O} is zero.
2. When U_{osm} is greater than P_{osm} (hypertonic urine), C_{osm} is greater than V; therefore, C_{H2O} is negative (also denoted as T^c_{H2O}).
3. When U_{osm} is less than P_{osm} (hypotonic urine), C_{osm} is less than V, and C_{H2O} is positive.

This relationship is depicted further in Figure 1-10.

The excretion of hypertonic urine has the net effect of returning solute-free water to the organism and thereby dilutes body fluids. In contrast, the excretion of hypotonic urine has the net effect of ridding the organism of solute-free water and thus concentrating body fluids. Urine osmolality alone does not give the volume of water added to or removed from the organism; the calculation of C_{H2O} or T^c_{H2O} better allows the quantitation of water balance.

A limitation of the equation is that it fails to predict clinically important alterations in tonicity and serum sodium concentration because it factors in urea. Urea is an important component of urine osmolality, but does not establish transcellular osmotic gradients because it readily crosses cell membranes. Consequently, urea influences neither the serum sodium concentration nor the release of vasopressin, and its inclusion in urine osmolality does not predict changes in serum sodium. This is better reflected if specifically electrolyte-free water clearance ($C_{H2O}[e]$) is measured. In this formulation, the serum osmolality is replaced by serum sodium and urine osmolality by $U_{Na} + U_K$.

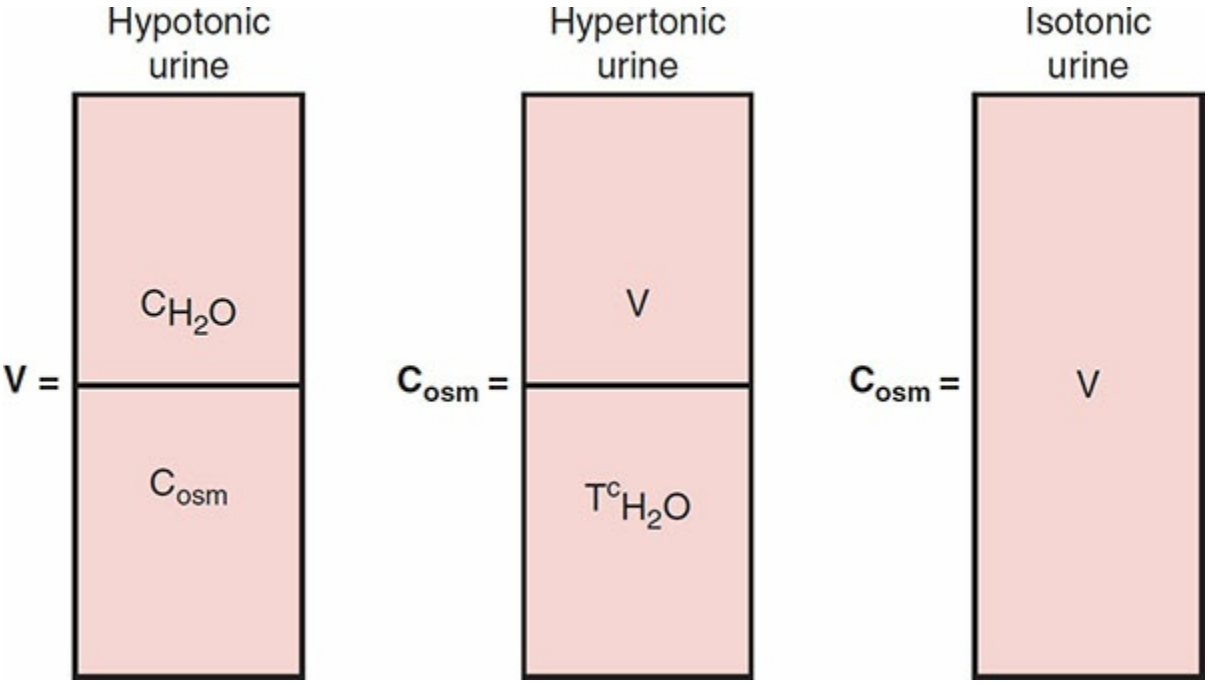


Figure 1–10 Relationship between urine flow (V), C_{osm}, CH_2O, and T^c_{H2O} in hypotonic, hypertonic, and isotonic urine.

Therefore:

$$C_{H_2O}(e) = V\left(1 - \frac{U_{NA} + U_K}{P_{NA}}\right) \quad (1.2)$$

If a patient's $U_{Na} + U_K < P_{Na}$, then $C_{H2O}(e)$ is positive, a process that will raise the plasma concentration of sodium. Conversely, if $U_{Na} + U_K > P_{Na}$, then $C_{H2O}(e)$ is negative, a process that tends to lower the serum concentration of sodium.

RELATIONSHIP BETWEEN DAILY SOLUTE LOAD, RENAL CONCENTRATING CAPACITY, AND DAILY URINE VOLUME

The ingestion of a diet containing average amounts of sodium (150 mmol/day) and protein (70 g/day) has to dispose of approximately 600 mOsm of solutes per day. The daily volume of urine in which this solute is excreted depends on fluid intake. The 600 mOsm can be excreted in 6 L of urine with an osmolality of 100 mOsm/kg H_2O if the daily fluid intake is generous. If water ingested is limited and renal concentrating capacity is intact, then the 600 mOsm solute load can be excreted in 500 mL of urine

with an osmolality of 1,200 mOsm/kg H_2O.

This flexibility in daily urine volumes for a given solute load is limited if renal concentrating ability is impaired. For example, if the maximal renal concentrating ability is reduced to 300 mOsm/kg H_2O, then the 600 mOsm of solute obligates 2 L of urine per day to maintain total body solute. The 600 mOsm of daily solute requires 10 L of urine per day with a more severe concentrating defect that does not allow urine to be concentrated above 60 mOsm/kg H_2O.

In terms of water conservation, the kidney's ability to increase urine osmolality from 60 to 300 mOsm/kg H_2O is quantitatively more important than its ability to increase urine osmolality from 300 to 1,200 mOsm/kg H_2O. For example, with a daily solute load of 600 mOsm, a decrease in maximal urine osmolality from 1,200 to 300 mOsm/kg H_2O increases obligatory urine flow from .5 to 2.0 L/day. Thus, severe polydipsia and polyuria should not be observed even in the complete absence of the renal capacity to concentrate urine above plasma. However, for the same solute load, a further decrease in maximal urinary concentration from 300 to 60 mOsm/kg H_2O requires the excretion to increase from 2 to 10 L of urine/day. This degree of defect in water conservation obviously is associated with overt polyuria and polydipsia. In this setting, a severe water deficit and hypernatremia occur in the absence of an intact thirst mechanism and a large intake of water.

RENAL CAPACITY TO REABSORB SOLUTE–FREE WATER (T^C_{H2O}) VERSUS CAPACITY TO EXCRETE SOLUTE-FREE WATER (C_{H2O})

In quantitative terms, the normal kidney's ability to reabsorb T^c_{H2O} is more limited than its ability to excrete C_{H2O}. With maximal urine osmolality of 1,200 mOsm/kg H_2O and a daily urine volume of 500 mL, T^c_{H2O} can be calculated as follows:

$$C_{osm} = \frac{UV}{P} \text{ or } C_{osm} = \frac{1{,}200 \text{ mOsm/kg } H_2O \times 500 \text{ mL/day}}{300 \text{ mOsm/kg } H_2O}$$

$$C_{osm} = 2{,}000 \text{ mL/day} \tag{1.3}$$

$$T^c_{H_2O} = C_{osm} - V$$

$$T^c_{H_2O} = 2{,}000 - 500 \text{ mL/day}$$

$$T^c_{H_2O} = 1{,}500 \text{ mL/day}$$

Thus, only 1,500 mL of solute-free water is returned to body fluids during this maximal antidiuresis. In contrast, with the same daily solute load of 600 mOsm, a minimal urine osmolality of 60 mOsm/kg H_2O, and a daily urine volume of 10 L, the renal capacity to excrete C_{H2O} is much greater than the capacity to return solute-free water (T^c_{H2O}) to the body. More specifically,

$$C_{osm} = \frac{UV}{P} \text{ or } \frac{60 \text{ mOsm/kg}}{300 \text{ mOsm/kg}} \times 10 = 2 \text{ L/day} \tag{1.4}$$

$$C_{H_2O} = V - C_{osm} \text{ or } 10 \text{ L} - 2 \text{ L} = 8 \text{ L/day}$$

Thus, with comparable solute loads and relatively maximal and minimal urine osmolalities, the T^c_{H2O} of 1.5 L/day is substantially less than the C_{H2O} of 8 L/day.

Thus, prevention of a total body water deficit is largely dependent on water intake as modulated by thirst. The thirst center appears to be closely associated anatomically with the osmoreceptor in the region of the hypothalamus. Defects in thirst response may involve either organic or generalized central nervous system (CNS) lesions and can lead to severe water deficit even in the presence of a normal concentrating mechanism. Of course, the water deficit occurs more promptly if renal concentrating ability is impaired as well.

Clinical Disorders of Urinary Concentration Causing Hypernatremic States

The renal concentrating mechanism represents the first defense against water depletion and hyperosmolality. A perturbation in any component of the concentrating mechanism, shown in Figure 1-1B, culminates in an

inability to maximally concentrate urine. Renal concentrating defects ensue when there is impairment in the generation of medullary hypertonicity either as a consequence of decreased delivery of solutes to the loop (diminished GFR) or inability to reabsorb NaCl in the loop of Henle (loop diuretics). Likewise, failure to render the collecting duct permeable to water because vasopressin is absent or the tubule is unresponsive to vasopressin also results in a renal concentrating defect. Thirst becomes a very effective mechanism for preventing further increases in serum sodium when renal concentration is impaired (20,21). The plasma osmolality threshold for thirst appears to be approximately 10 mOsm/kg H_2O above that of vasopressin release (Fig. 1-8). In fact, thirst is so effective that even patients with complete diabetes insipidus avoid hypernatremia by fluid intake in excess of 10 L/day. Therefore, hypernatremia supervenes only when hypotonic fluid losses occur in combination with a disturbance in water intake (22). This is most commonly seen in the aged (with an alteration in level of consciousness), the very young (with inadequate access to water), or a rare subject (with a primary disturbance in thirst).

Hypernatremia can develop with either low, normal, or, more rarely, high total body sodium, as shown in Figure 1-11 (23).

HYPERNATREMIA IN PATIENTS WITH LOW TOTAL BODY SODIUM

Patients who sustain losses of both sodium and water, but with a relatively greater loss of water, are classified as having hypernatremia with low total body sodium. Such patients exhibit the signs of hypovolemia such as orthostatic hypotension, tachycardia, flat neck veins, poor skin turgor, and dry mucous membranes. The causes that underlie the hypovolemic state are similar to those that cause hypovolemic hyponatremia. The effect on serum sodium is determined by the failure to ingest water (hypernatremia) or excessive free water intake (hyponatremia). Extrarenal loss of hypotonic fluid can occur either through the skin because of profuse sweating in a hot and/or humid environment or, more frequently, from the gastrointestinal tract in the form of diarrhea. Lactulose-induced diarrhea leading to hypernatremia appears to be common, although primarily recognized in children. Urine osmolality is high (usually >800 mOsm/kg H_2O) and urinary sodium concentration is low (<10 mEq/L) because the renal water and sodium conserving mechanisms operate normally in these patients. Hypotonic losses also can occur by the renal route during a loop diuretic–

induced hypotonic diuresis or an osmotic diuresis with either mannitol, glucose, or as is not uncommon, urea in the setting of excessive protein supplementation. Elderly patients with partial urinary tract obstruction can excrete large volumes of hypotonic urine. The urine with such obstruction is hypotonic or isotonic, and the urinary sodium concentration is greater than 20 mEq/L. As glucose and mannitol enhance osmotic water movement from the intracellular fluid to the ECF compartment, these patients may have a normal or even low serum sodium concentration in spite of serum hypertonicity.

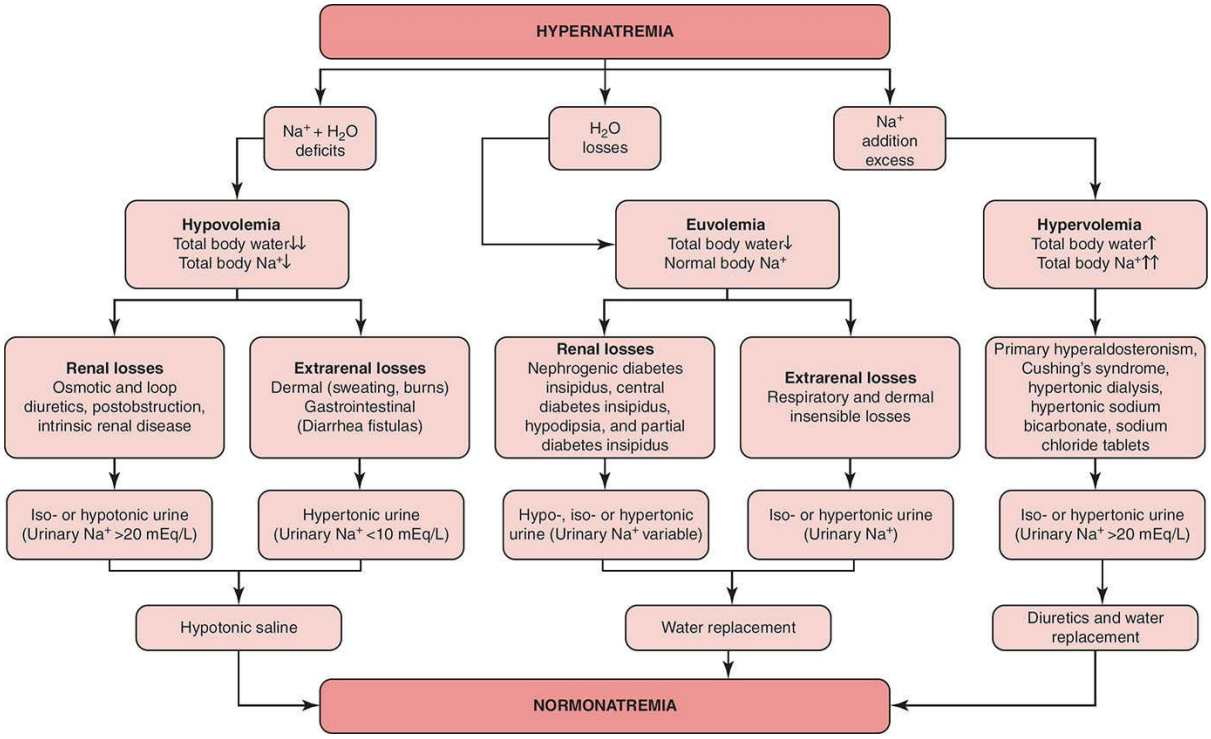


Figure 1–11 Diagnostic and therapeutic approach to the hypernatremic patient. (From Berl T, Kumar S. Disorders of water balance. In: Johnson RJ, Feehally J, eds. *Comprehensive Clinical Nephrology*. St. Louis: CV Mosby; 2000:3–9, with permission.)

HYPERNATREMIA IN PATIENTS WITH NORMAL TOTAL BODY SODIUM

Loss of water without sodium does not lead to clinically significant volume contraction unless the water losses are massive. Therefore, patients with hypernatremia secondary to water loss appear to be euvolemic with normal total body sodium. The extrarenal sources of such water losses are the skin and respiratory tract. A high environmental temperature as well as a febrile or hypermetabolic state can cause considerable water losses.

Hypernatremia supervenes if such hypotonic losses are not accompanied by appropriate water intake. Urine osmolality is very high, reflecting an intact osmoreceptor–vasopressin–renal response. Urinary sodium concentration varies according to the patient's sodium intake.

More frequently, the losses of water are of renal origin, as in diabetes insipidus. Diabetes insipidus is a polyuric disorder characterized by high rates of electrolyte-free water excretion. Hypernatremia supervenes when these losses are not appropriately replaced. Depending on whether the water losses are caused by a failure to secrete vasopressin or renal resistance to the hormone, the diabetes insipidus is designated as being central or nephrogenic, respectively.

Central Diabetes Insipidus

Failure to normally synthesize or secrete vasopressin limits maximal urinary concentration and causes varying degrees of polyuria and polydipsia, depending on the severity of the disease. The causes of central diabetes insipidus are listed in Table 1-1.

In a survey of 79 children and young adults, the disease was idiopathic in 52%, with a significant number having tumors and Langerhans cell histiocytosis. Most had magnetic resonance imaging (MRI) findings and some had thickening of the pituitary stalk that may reflect lymphocytic infiltration as part of an autoimmune process. The probability of also developing anterior pituitary hormone deficiency was 80% in the group that had tumors, compared to 50% in subjects with idiopathic diabetes insipidus (24,25). The disease may rarely be inherited. Families with an autosomal dominant inheritance pattern have been described (26). Mutations in the coding region of the gene in all three exons have been described affecting one allele. These mutations are in the signal protein and neurophysin. Most are missense mutations, but other mutations have been described as well (26).What is peculiar about this inherited form of central diabetes insipidus is that the onset of symptoms is delayed for several months after birth and sometimes even longer. It appears that the mutant hormone forms complexes with the native hormone and the accumulation of these complexes in the endoplasmic reticulum causes progressive loss of vasopressin-producing neurons (27). There is also a rare inherited autosomal recessive form of central diabetes insipidus that occurs in association with diabetes mellitus, optic atrophy, and deafness (Wolfram syndrome). The syndrome appears to result from mutations in region PC 16 of chromosome 4, which codes for a protein expressed in

various tissues (28).

Head trauma, hypophysectomy, and neoplasms, either primary or metastatic (mainly from lung and breast tumors), constitute most of the other causes. Other etiologic factors include encephalitis, sarcoidosis, eosinophilic granuloma, and histiocytosis. Finally, central diabetes insipidus has been described following development of cerebral edema in 11 postoperative hyponatremic women (29).

Clinical Features

Polyuria and polydipsia are the hallmarks of central diabetes insipidus and must be considered in the differential diagnosis of any patient who presents with such symptoms. As illustrated in Figure 1-10, polyuria can occur from a solute diuresis, in which case C_{osm} is increased and the urine osmolality is greater than 300 mOsm/kg. A diagnosis of central (vasopressin-deficient) diabetes insipidus should be considered when polyuria is caused by an increase in C_{H2O} and urine osmolality is less than 150 mOsm/kg. Urine flow can range between 3 and 15 L/day, depending on the severity of the disease. The disorder frequently has an abrupt onset and occurs with equal frequency in both sexes. Although the time of onset is extremely variable, it is rare in infancy and is most frequent in the 10- to 20-year age group. Patients with central diabetes insipidus often have a predilection for cold water. Nocturia frequently is marked because there is little diurnal variation in the polyuria. Bladder capacity may be increased in untreated patients, however; consequently, nocturia may not be a prominent symptom. Nevertheless, nocturia is frequent generally, and sleep deprivation commonly leads to fatigue and irritability. Patients with central diabetes insipidus do not develop hypernatremia if the thirst mechanism is intact and water is available; thus, they have no symptoms except for the inconvenience associated with marked polyuria and polydipsia. However, severe and even life-threatening hypernatremia can supervene with concomitant hypodipsia, no access to water, or an illness that precludes adequate water intake.

Table 1–1 Causes of Central Diabetes Insipidus

Hereditary
Autosomal dominant
Autosomal recessive (Wolfram syndrome)

Acquired
- Head trauma, skull fracture, and orbital trauma
- Posthypophysectomy
- Suprasellar and intrasellar tumors
- Primary (suprasellar cyst, craniopharyngioma, pinealoma, meningioma, and glioma)
- Metastatic (breast or lung cancer, leukemia, and lymphomas)

Granulomas
- Sarcoid
- Wegener granulomatosis
- Tuberculosis
- Syphilis

Histiocytosis
- Eosinophilic granuloma
- Hand–Schüller–Christian disease

Infections
- Encephalitis
- Meningitis
- Guillain–Barré syndrome

Vascular
- Cerebral aneurysm
- Cerebral thrombosis or hemorrhage
- Sickle cell disease
- Postpartum necrosis (Sheehan syndrome)
- Pregnancy (transient)

From Levi M, Berl T. Water metabolism. In: Gonick HC, ed. *Current Nephrology*. Vol 5. Chicago: Year Book Medical Publishers; 1982:23, with permission.

Diagnosis

The development of severe polyuria and polydipsia (>6–8 L/day) in an adult patient who does not have diabetes mellitus (the most common cause of a solute diuresis) indicates the possibility of either a failure of vasopressin release (central diabetes insipidus) or excessive water intake (dipsogenic diabetes insipidus or primary polydipsia), or a failure of the collecting duct to respond to vasopressin (nephrogenic diabetes insipidus).

The differential diagnosis between central diabetes insipidus and primary polydipsia may be very difficult. Plasma vasopressin levels are

diminished in both circumstances. This is caused by impaired synthesis or secretion of vasopressin in central diabetes insipidus. Thus, these patients have polydipsia secondary to impaired renal water conservation in the absence of AVP. In contrast, the patient with primary polydipsia ingests large amounts of fluids, which physiologically suppress endogenous AVP release, resulting in the urinary excretion of the large volume of ingested water. Thus, the patient with central diabetes insipidus has polydipsia because of polyuria, whereas the individual with primary polydipsia has polyuria because of polydipsia. Abnormalities in the hypothalamic-pituitary region can be seen in a majority of patients with central diabetes insipidus with the use of the computed tomography scan. MRI may improve the sensitivity further. Normally, on T1-weighted images, the posterior pituitary produces a bright signal that is indistinguishable from fatty tissue, but this signal is lost in patients with central diabetes insipidus (30).

The patient's history can be helpful in making the differential diagnosis. Whereas the patient with central diabetes insipidus has an abrupt onset of polyuria and polydipsia, the patient with primary polydipsia has a more vague history of the onset of these symptoms. The latter patients also may have a history of considerable variation in water intake and urine output on an hour-to-hour or day-to-day basis, whereas the patient with central diabetes insipidus has a very consistent need for water intake. Large variations in water intake, in the patient whose intakes and outputs are measured, therefore are a clue to the diagnosis of compulsive water drinking. Nocturia is more severe and frequent in subjects with central diabetes insipidus. Finally, the previously noted preference for ice water usually is not described by subjects with primary polydipsia. These patients also may have a history of psychiatric disorders and not infrequently are women during menopause. A plasma osmolality below 270 mOsm/kg H_2O strongly suggests primary polydipsia because of the modest positive fluid balance, whereas the patient with central diabetes insipidus is generally in modest negative fluid balance. Thus, a sodium greater than 143 mm/L or plasma osmolality above 295 mOsm/kg H_2O essentially excludes primary polydipsia and suggests central diabetes insipidus. Although the differentiation between central and dipsogenic diabetes insipidus in their classic forms may pose no difficulties, the correct diagnosis is frequently difficult to make when the defect in vasopressin release is partial. A fluid deprivation test may provide the most reliable information regarding the assessment of these polyuric disorders (Fig. 1-12). Fluid deprivation must be instituted with careful

monitoring of body weight and vital signs, because the patient with central diabetes insipidus may rapidly develop a severe negative fluid balance. The test is stopped when body weight decreases by more than 3%, the patient develops orthostatic blood pressure changes when the serum sodium is greater than 145 mmol/L, or the urine osmolality reaches a plateau in three consecutive hourly collections. This period of fluid deprivation is followed by the injection of 5 units of aqueous vasopressin or 1 μg of DDAVP subcutaneously. Normal subjects require 16 to 18 hours to achieve a mean maximum urine osmolality of approximately 1,000 to 1,200 mOsm/kg and the administration of vasopressin causes no further increase in their urine osmolality. This suggests that the dehydration test has maximally stimulated endogenous vasopressin release. One might surmise, therefore, that fluid deprivation readily discriminates between those with a normal neurohypophyseal system, such as those with primary polydipsia, and the patient with central diabetes insipidus. As illustrated in Figure 1-12, this is not always the case. Observations of normal subjects who have drunk large daily volumes of water, however, have demonstrated a blunted response to vasopressin (31). A decrease in medullary tonicity occurs as a result of an increase in medullary blood flow and is associated with the diminution in renal concentrating capacity. For this reason, patients with primary polydipsia may demonstrate submaximal concentrating ability after fluid deprivation, but their urine osmolality still generally exceeds 300 mOsm/kg. There is however, no further increase with exogenous vasopressin because endogenous vasopressin secretion is maximal with fluid deprivation. This serves to differentiate such patients from those with central diabetes insipidus, whose urine osmolality substantially increases (>10%) following the administration of vasopressin (Fig. 1-12). The recognition of patients who have only a partial defect in AVP secretion is of particular importance. Urine osmolalities in these patients and those with primary polydipsia may be similar after fluid deprivation, but only the patient with partial diabetes insipidus will respond further to exogenous vasopressin. If exogenous vasopressin increases urine osmolality by more than 10% after fluid deprivation, a defect in AVP release is probably present. Only patients with complete diabetes insipidus may demonstrate overt clinical symptoms of polyuria and polydipsia, whereas patients with partial central diabetes insipidus may remain asymptomatic. The measurement of urinary AQPs has been suggested to differentiate various forms of diabetes insipidus (32), and more specifically to differentiate psychogenic polydipsia for central diabetes insipidus (33). Urinary AQP_2 was decreased

in subjects with central deficiency AVP, but not in those with psychogenic polydipsia. The clinical applicability of this test is limited at this time.

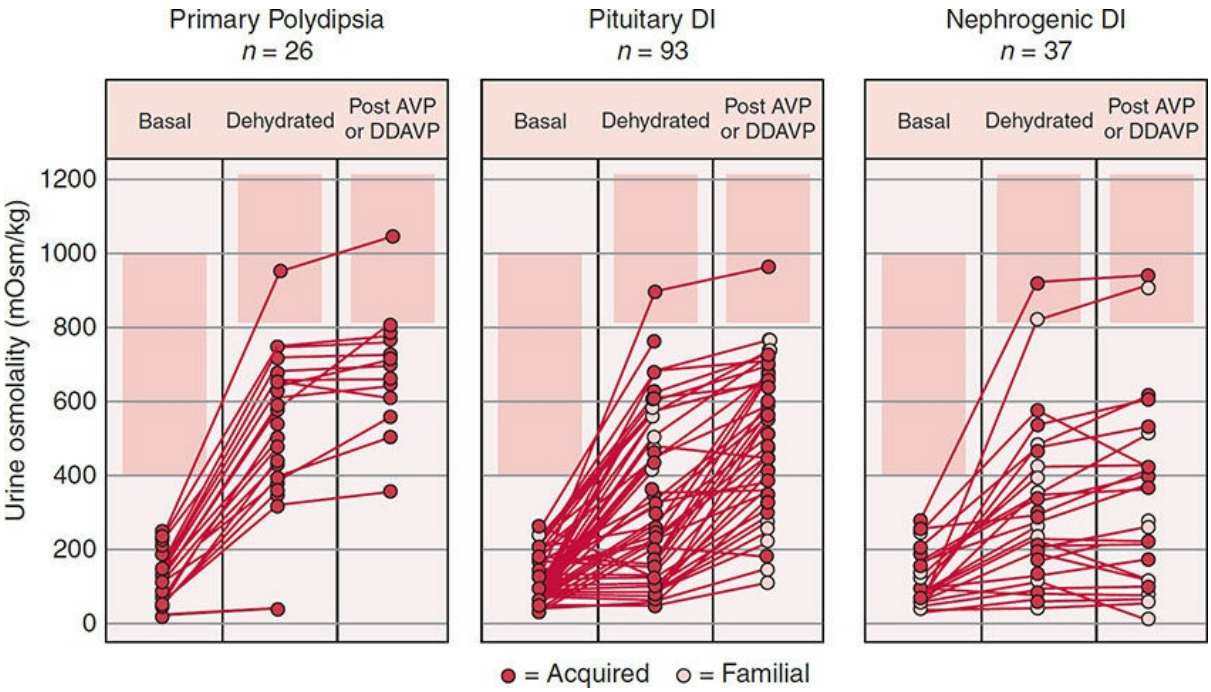


Figure 1–12 Effects of fluid deprivation and subsequent arginine vasopressin (AVP) (Pitressin) administration on urine osmolality in 156 patients with polyuria of diverse causes. The shaded area indicates the range of values in healthy adults. Note that although AVP responses are greater in patients with central (neurogenic) diabetes insipidus (DI), the overlap between the three groups is significant. (From Brenner BM. *The Kidney*. 8th ed. Philadelphia; Saunders Elsevier; 2008, with permission.)

The above described water deprivation test followed by exogenous AVP administration is useful in the differential diagnosis of polyuric disorders in 95% of cases; it is time consuming and requires considerable patient cooperation. Occasionally, the diagnosis of central diabetes insipidus needs to be made more promptly or in acutely ill subjects. An examination of the relationship between plasma osmolality (excluding glucose and urea) and urine osmolality can be helpful. The finding of a low urine osmolality as plasma tonicity rises during a brief period of water withdrawal suggests the diagnosis of central diabetes insipidus.

Measurements of circulating AVP can serve as a valuable adjunct to the water deprivation test and in large measure confirmed the diagnosis reached by the dehydration test in most patients. The incorporation of vasopressin measurements by a sensitive radioimmunoassay may complement and refine the accuracy of previously available tests in the differential diagnosis of polyuric syndromes, but is not essential to the establishment of the various diagnoses and is not routinely measured.

Treatment of Central Diabetes Insipidus

Patients with central diabetes insipidus do not develop hypernatremia if the thirst mechanism is intact and water is available; thus, they have no symptoms except for the inconvenience associated with marked polyuria and polydipsia. Nonetheless, studies in acute care settings have clearly shown that patients who present with (34) or acquire (35) hypernatremia have a higher risk for mortality.

Both AVP replacement and pharmacologic agents are available for the treatment of central diabetes insipidus (Table 1-2). In acute settings, such as after hypophysectomy, the aqueous vasopressin (Pitressin) preparation is preferable. Its short duration of action allows for more careful monitoring and decreases the likelihood of complications such as water intoxication.

Table 1–2 Therapeutic Regimens for the Treatment of Diabetes Insipidus

	Drug	Dose
Complete central diabetes insipidus	DDAVP	10–20 μg intranasally q12–24 h
	Oral	100–800 μg/d
Partial central diabetes insipidus	DDAVP	As above
	Aqueous vasopressin	5–10 units SQ q4–6 h
	Chlorpropamide	250–500 mg/d
	Clofibrate	500 mg t.i.d.–q.i.d.
	Carbamazepine	400–600 mg/d
NDI	Thiazide diuretics	—
	Amiloride (for lithium-related NDI)	5 mg q.i.d.
Gestational diabetes insipidus	DDAVP	As above

DDAVP, desmopressin acetate; NDI, nephrogenic diabetes insipidus.
Reprinted from Thurman JB, Berl T. Therapy in nephrology and hypertension. In: Wilcox JN, ed. *Therapy in Nephrology and Hypertension*. 3rd ed. Philadelphia: Saunders; 2008:337–352, with permission from Elsevier.

A modification of the natural vasopressin molecule to form

desmopressin acetate (DDAVP) has resulted in a compound with prolonged antidiuretic activity (6–24 hours) and virtual elimination of V_1 vasopressor receptor activity (antidiuretic to pressor ratio of approximately 2,000:1) as compared with the natural hormone AVP (duration of action of 2–4 hours and antidiuretic to pressor ratio of approximately 1:1). Substitution of D-arginine for L-arginine at position 8 resulted in a peptide DAVP with diminished vasopressor activity, and deamination of the homocysteine at position 1 gave rise to a second peptide, with enhanced antidiuretic pressor activity and prolonged duration of action. Desmopressin acetate is administered intranasally in a dosage ranging from 10 to 20 μg every 8 to 12 hours. The drug has eliminated the need for previously employed long-acting vasopressin in oil. There are considerable individual variations in the required dosage, but most patients require twice-daily administration for good control of polyuria. Desmopressin acetate also can be administered intravenously or subcutaneously during periods of respiratory illness or surgery.in doses between 1 and 4 g. It is active orally in large doses also (between 50 and 800 μg) (34).

Large doses of DDAVP may cause transient headaches, nausea, and a slight increase in blood pressure; these symptoms disappear if the dosage is reduced. Nasal congestion, mild abdominal cramps, and vulval pain have occurred rarely. These patients need careful monitoring of water intake and serum sodium to avoid development of hyponatremia. In fact, there are increasing reports of cases of hyponatremia in patients on these agents, particularly when used for other indications such as von Willebrand disease (35) and enuresis (36).

Intranasal DDAVP currently is the treatment of choice for partial or complete central diabetes insipidus. However, alternatives to hormone replacement may be helpful at times. With dilute urine of fixed low osmolality, the urine volume is determined by the solute load requiring excretion. A reduction in salt and protein in the diet therefore will reduce the major urinary solutes and thus the volume of urine necessary to accommodate their excretion. Moreover, a number of pharmacologic agents with antidiuretic properties are used; the hypoglycemic agent chlorpropamide (Diabinese) is the most commonly employed. Its antidiuretic effects are manifested only if some vasopressin is present; therefore, it is useful only in partial diabetes insipidus. A trial of 250 mg every day or twice a day may be offered to patients with partial central diabetes insipidus and at least 7 days allowed for an effect to occur. The anticonvulsant carbamazepine (Tegretol) has caused antidiuresis in subjects with central diabetes insipidus. A combination of chlorpropamide

and carbamazepine has been found to provide an effect that could be synergistic. Clofibrate also has been used to treat partial central diabetes insipidus. At present, however, none of these approaches can be recommended over intranasal DDAVP.

Congenital Nephrogenic Diabetes Insipidus

Congenital nephrogenic diabetes insipidus is a rare hereditary disorder in which the renal tubule is insensitive to vasopressin (37). The disease has been described in various patterns, including the X-linked form, and autosomal recessive and even an autosomal dominant form. The most common variety is the X-linked whose complete form manifests itself in males with females expressing variable degrees of polyuria and polydipsia. In 85% of patients, the disease is a consequence of mutations on the V_2 receptor, resulting in a loss of function (38). More than 180 mutations of the V_2 receptor in chromosome region Xq28 have been identified (39). Half are missense mutations but other types of mutations occur as well. A significant number of the mutant receptors have defective intracellular trafficking (37). The autosomal recessive form of congenital nephrogenic diabetes insipidus is to a mutation in the AQP_2 water channel and accounts for approximately 15% of disease (40). At least 30 disease-causing mutations have been identified, most of them of the missense type. As was the case with the V_2 receptor mutant, misrouting of the AQP_2 mutant protein has been described in this setting (41). Mutations at the carboxy terminal of AQP_2 can cause a rare autosomal dominant form of congenital nephrogenic diabetes insipidus (42). A modest concentrating defect is present also in humans deficient in the AQP_1 water channel (43). Finally, knockout mice lacking AQP_3 and/or AQP_4 also fail to maximally concentrate their urine (44).

Clinical Manifestations

Distinct phenotype differences among the various genotypes have not been completely described. The most complete clinical description is available for the X-linked form. Although the disease is most probably inborn, the diagnosis of this form of congenital nephrogenic diabetes insipidus is usually not made until the infant presents with hypoosmolar urine in the face of severe dehydration, hypernatremia, vomiting, and fever. Unlike some of the females, who have partial responsiveness to vasopressin,

males with the full-blown complete form of this disorder do not elaborate hypertonic urine even in the face of severe dehydration. The impaired growth and occasional mental retardation that occur in these cases, if not treated with adequate fluids, are most likely the result of repeated episodes of dehydration and hypernatremia rather than being integral components of the disease. Hydronephrosis is common in these patients perhaps because of voluntary retention of large volumes of urine with subsequent vesicoureteral reflux.

Treatment of Congenital Nephrogenic Diabetes Insipidus

Neither vasopressin nor other pharmacologic agents that potentiate its action or stimulate its release (e.g., chlorpropamide) are effective in concentrating the urine of patients with congenital nephrogenic diabetes insipidus. Consequently, an intact thirst mechanism is indispensable for the maintenance of good hydration in children with this disorder, as is careful monitoring of fluid balance. Children with this disorder who need rehydration should receive hypotonic (2.5%) rather than isotonic (5%) glucose solutions because the excretion of solute requires further water losses. Glucosuria may occur with the latter solution and thus aggravate fluid losses.

Limitation of oral solute intake (low-sodium diet) also may lead to a decrease in urine flow in patients with nephrogenic diabetes insipidus. Thiazide diuretics, which inhibit sodium reabsorption in the cortical diluting segment of the nephron, have met with some success in the management of these patients. The ability of thiazides to diminish sodium reabsorption in this water-impermeable portion of the nephron would by itself decrease C_{H2O} but not urine flow. It seems most likely that the decrease in urine flow is secondary to the sodium loss and ECF volume contraction. ECF volume depletion in turn decreases GFR and increases proximal tubular sodium and water reabsorption. These secondary effects of the diuretic agent then decrease urine flow. The ECF volume contraction can be maintained with a low sodium intake after discontinuance of the diuretic, so that the therapy still remains effective. The addition of amiloride to hydrochlorothiazide may provide added benefit. Nonsteroidal antiinflammatory drugs have been found to be effective, and in this regard, tolmetin appears to be particularly well tolerated in children. It should be noted that none of these modalities results in the elaboration of hypertonic urine. Even an increase in urine osmolality from 50 to 200 mOsm/kg H_2O is very important, however,

because it significantly reduces obligatory urine loss from 10 to 12 L/day to a tolerable 3 to 4 L/day. Such a change in urine flow also minimizes the dilatation of the urinary tract. An intriguing new approach involves the use of cell-permeable vasopressin antagonists as chaperones that facilitate the folding of the mutant protein retained in the endoplasmic reticulum and increase the expression of the cell surface (45). In one study of subjects with nephrogenic diabetes insipidus, this approach resulted in a decrease in urine flow from 12 to 8 L with a modest increase in urinary osmolality (46).

Acquired Nephrogenic Diabetes Insipidus

The acquired form of nephrogenic diabetes insipidus is much more common than the congenital form of the disease, but it is rarely severe. In fact, although maximal concentrating ability is impaired in this disorder, the ability to elaborate hypertonic urine usually is preserved. Nocturia, polyuria, and polydipsia may occur in this acquired form of nephrogenic diabetes insipidus, but the urine volumes generally are less (<3–4 L/day) than those observed with complete central diabetes insipidus, psychogenic water drinking, or congenital nephrogenic central diabetes insipidus. The more common causes of acquired nephrogenic diabetes insipidus are listed in Table 1-3.

Chronic Renal Disease

A defect in renal concentrating capacity is a consistent accompaniment of most forms of advanced renal failure. Thus, chronic renal disease constitutes a form of acquired nephrogenic diabetes insipidus. Advanced renal insufficiency of any cause can cause a vasopressin resistance associated with hypotonic urine (47).

In some forms of kidney disease, listed in Table 1-3, vasopressin unresponsiveness can occur at a stage when GFR is not markedly diminished. The occurrence of a profound diuresis in association with a concentrating defect in glomerular diseases of the kidney is rare, and in general, a close correlation exists between GFR and maximal urine osmolality.

The causes of the defect in renal concentrating capacity associated with chronic renal failure are probably multiple (48). These include: (a) a disruption of inner medullary structures or local alterations in medullary blood flow as is seen in tubulointerstitial diseases, sickle cell disease, and

analgesic nephropathy; (b) an impairment in sodium chloride transport out of the thick ascending limb of Henle's loop, a process that limits maximal interstitial tonicity; and (c) an increase in solute excretion in the remaining few functioning nephrons, an adaptive response to the need to excrete the same solute load as the normal kidney. Solute diuresis in normal humans may cause isotonic urine in the presence of maximal amounts of vasopressin. However, none of these pathogenic mechanisms alone can explain the observation that vasopressin-resistant hypotonic urine may be found in patients with advanced renal failure (47). If the assumption is made that even in the absence of a countercurrent system the tonicity of the renal medulla is never less than that of plasma, a failure of complete osmotic equilibration between the collecting duct and medullary interstitium must occur to explain vasopressin-resistant hypotonic urine. One possibility is that the response to AVP of the collecting duct membranes in the damaged kidney is submaximal as a result of selective downregulation of the V_2 receptor (49). In a model of 5/6 nephrectomy-induced renal failure, a decrease in collecting duct AQP_2 and AQP_3 expression was reported (50). A similar decrement in AQP_2 has been described in obstructive uropathy and during recovery from acute tubular necrosis (Fig. 1-13).

Table 1–3 Causes of Acquired Nephrogenic Diabetes Insipidus

Chronic renal disease
Polycystic disease
Medullary cystic disease
Ureteral obstruction
Amyloidosis
Advanced renal failure of any etiology
Electrolyte disorders
Hypokalemia
Hypercalcemia
Drugs
Alcohol
Phenytoin
Lithium
Demeclocycline
Acetohexamide
Tolazamide

- Glyburide
- Propoxyphene
- Amphotericin
- Foscarnet
- Methoxyflurane
- Norepinephrine
- Vinblastine
- Colchicine
- Gentamicin
- Methicillin
- Isophosphamide
- Angiographic dyes
- Osmotic diuretics
- Furosemide and ethacrynic acid

Sickle cell disease

Dietary abnormalities
- Excessive water intake
- Decreased sodium chloride intake
- Decreased protein intake

Miscellaneous
- Gestational diabetes insipidus

Recognition of the renal concentration defect is foremost in the therapeutic approach. If the maximal renal concentrating capacity of a patient with chronic renal failure is 300 mOsm/kg H_2O and the daily solute load is 600 mOsm, a urine volume of 2 L/day is necessary to excrete the solute load. The patient's fluid intake, including 500 mL for insensible losses, must therefore be at least 2,500 mL/day. Thus, if the patient is ill and cannot ingest fluids for several days, severe water depletion can occur because of the failure of the kidney to concentrate the urine. Recognition of the subclinical concentrating defect, which can emerge as an important clinical problem during an acute illness, therefore is pivotal in the long-term management of patients with chronic renal disease.

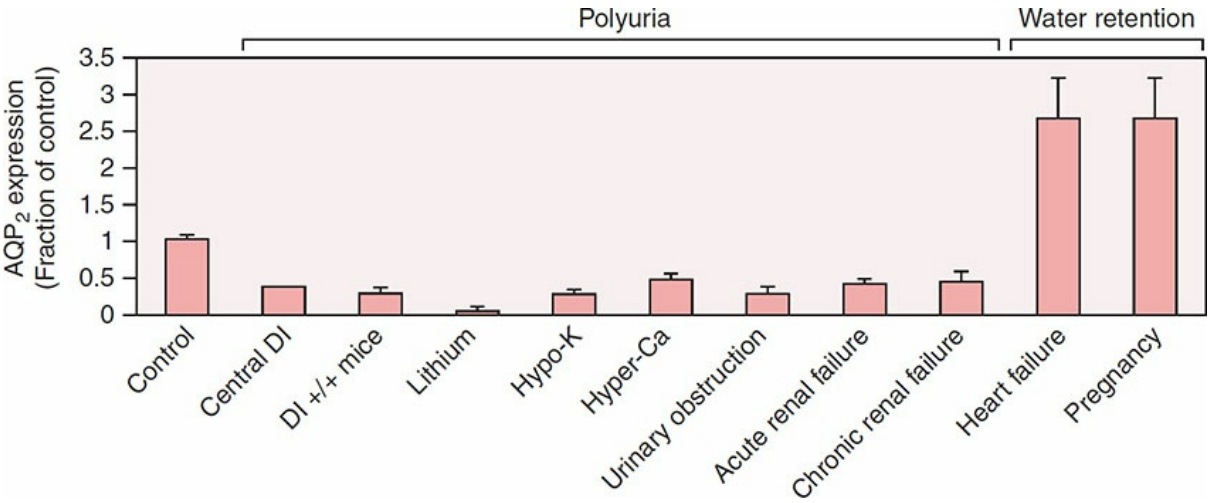


Figure 1–13 Changes in AQP_2 expression seen in association with different water balance disorders. Levels are expressed as a percentage of control levels (*leftmost bar*). AQP_2 expression is reduced, sometimes dramatically, in a wide range of hereditary and acquired forms of diabetes insipidus characterized by different degrees of polyuria. Conversely, congestive heart failure and pregnancy are conditions associated with increased expression of AQP_2 levels and excessive water retention. (From Neilson SK, Knepper MK, Kwon T, et al. Urine concentration and dilution. In: Schrier RW, ed. *Diseases of the Kidney and Urinary Tract*. Vol 1. 7th ed. New York: Lippincott Williams & Wilkins; 2000:128, with permission.)

Electrolyte Abnormalities

Hypokalemia of any cause has long been known to cause polyuria and a reversible form of a vasopressin-resistant renal concentrating defect. The pathogenesis is multifactorial. Hypokalemia stimulates water intake and reduces interstitial tonicity, as a consequence of a decreased Na+/Cl– reabsorption in the thick ascending limb. Hypokalemia also decreases intracellular cAMP accumulation and causes a reduction in vasopressin-sensitive AQP_2 expression (51) (Fig. 1-13).

Hypercalcemia also impairs urinary concentrating ability, resulting in mild polydipsia. The pathophysiology is also multifactorial and includes a reduction in medullary interstitial tonicity caused by decreased vasopressin-stimulated adenylate cyclase in the thick ascending limb and a defect in adenylate cyclase activity with decreased AQP_2 expression in the collecting duct (52) (Fig. 1-13).

Pharmacologic Agents

Various pharmacologic agents have also been found to impair the renal capacity to concentrate urine (Table 1-3). By virtue of its widespread use

in the treatment of affective disorders, lithium has emerged as perhaps the most common cause of congenital nephrogenic diabetes insipidus, affecting as many as 50% of patients on the drug. Lithium decreases vasopressin-stimulated water transport in the perfused cortical collecting duct. This is most likely a consequence of inhibition of adenylate cyclase and cAMP generation (37). A marked downregulation of AQP_2 and AQP_3 has been described in lithium-treated rats (53,54). Lithium may also increase the expression of cyclooxygenase-2 and lead to increasing renal prostaglandins which contribute to polyuria (55). The concentrating defect may be persistent and not entirely reversible.

Demeclocycline is another drug that causes nephrogenic diabetes insipidus. Because it is better tolerated than lithium, it is a better choice agent to treat the syndrome of inappropriate AVP release. However, the recently approved orally active tolvaptan may be preferable for syndrome of inappropriate antidiuretic hormone (SIADH). The precise cellular mechanism whereby demeclocycline causes this effect has not been elucidated.

Sickle Cell Anemia

A renal concentrating defect is a common accompaniment of sickle cell anemia and sickle cell trait. Sickling of red blood cells in the hypertonic medullary interstitium with occlusion of the vasa recta appears to cause inner medullary and papillary damage. Microradioangiographic studies have failed to demonstrate vasa recta blood flow in patients with sickle cell disease. The resultant medullary ischemia may impair sodium chloride transport in the ascending limb and thus diminish medullary tonicity. Transfusions of normal blood have been shown to restore renal concentrating capacity in children, thus indicating that the sickled red blood cells have a role in the defect. Medullary infarcts occur with more prolonged disease, and the concentrating defect is no longer reversible with transfusions. The diminished maximal urine osmolality also occurs in sickle cell anemia in association with papillary edema, thus providing a situation analogous to experimental papillectomy.

Dietary Abnormalities

As noted in the discussion of primary polydipsia, excessive water intake culminates in an impairment of maximal urinary concentration. In a recent experimental study, primary polydipsia did not decrease medullary

interstitial tonicity, but an alteration in the cellular action of vasopressin was shown to be associated with downregulation of AQP_2 (56). A marked restriction in sodium chloride intake also impairs the urinary concentrating mechanism. A similar defect is encountered in states of severe protein restriction. Because urea and sodium chloride account for virtually all interstitial tonicity, their decreased availability may account in large part for the observed defect. A defect in water reabsorption related to a decrement in AQP expression has been invoked (57).

Gestational Diabetes Insipidus

An acquired form of diabetes insipidus has been described in pregnancy that is AVP unresponsive by. An increase in circulating vasopressinase leading to excessive catabolism explains vasopressin resistance (58). Such patients can develop severe hypernatremia. However, because DDAVP is not affected by vasopressinase, this agent can reduce urine flow and can serve as a diagnostic tool of this entity.

HYPERNATREMIA IN PATIENTS WITH INCREASED TOTAL BODY SODIUM

Hypernatremia with increased total body sodium is the least common type of hypernatremia and is usually caused by exogenous administration of hypertonic sodium-containing solutes (Table 1-4). Hypernatremia supervenes during resuscitative efforts with hypertonic sodium bicarbonate, inadvertent intravascular infusion of hypertonic saline in therapeutic abortions, inadvertent dialysis against a high sodium concentration dialysate, seawater drowning, and even after ingestion of large quantities of sodium chloride tablets. Patients with primary hyperaldosteronism and Cushing syndrome have slight, clinically unimportant elevations in serum sodium concentration. As expected, patients with hypernatremia and high total body sodium excrete generous quantities of the cation in their urine concentrate (Fig. 1-11).

Table 1–4 Therapeutic Hypertonic Solutions

Solute	Molecular Weight	Concentration (mg/dL)	Osmolality (mOsm/kg H_2O)	Typical Container Size (mL)	Use
Sodium chloride	58.5	3 5 20	1,026 1,711 6,845	500 500 250	Emergency treatment of hypotonic states; intraamniotic instillation for therapeutic abortion
Sodium bicarbonate	84.0	5 7.5	1,190 1,786	500 50	Treatment of metabolic acidosis, hyperkalemia, cardiopulmonary arrest

From Arieff AI, et al. Pathophysiology of hyperosmolar states. In: Andreoli TE, et al (eds): Disturbances in Body Fluid Osmolality. Bethesda, MD: American Physiology Society; 1977, with permission.

Signs and Symptoms of Hypernatremia

Hypernatremia always reflects a hyperosmolar state. The most prominent manifestations of hyperosmolar disorders are of a neurologic nature. These follow movement of water out of cells, resulting in cellular dehydration, particularly in the brain. The signs and symptoms of hypernatremia are listed in Table 1-5. Restlessness, irritability, depression of sensorium, lethargy, muscular twitching, hyperreflexia, and spasticity may occur and culminate in coma, seizures, and death. In children, the mortality of acute hypernatremia ranges between 10% and 70%, with a mean of approximately 45%. Unfortunately, neurologic sequelae are common even in survivors, affecting as many as two-thirds of the children. Mortality in chronic hypernatremia is approximately 10%. In adults, acute elevation of serum sodium above 160 mEq/L is associated with 75% mortality, whereas the mortality in chronic cases is approximately 60%. Recent studies in acute care settings have clearly shown that patients who present with (59) or acquire (60) hypernatremia have a higher risk of mortality. It must be pointed out, however, that hypernatremia frequently occurs in the adult in the setting of serious underlying diseases, which may be the primary cause of the high mortality. The sequelae of hypernatremia in adults have not yet been studied systematically.

The signs and symptoms of hypernatremia are most likely related to a variety of anatomic derangements. The loss of volume and shrinkage of brain cells associated with the hyperosmolar states cause tearing of cerebral vessels. In addition to these gross anatomic changes, the brain sustains alterations in the composition of water and solutes that may be of great importance in the pathophysiology of the symptoms of hypernatremia (61). These are responses designed to regulate volume and restore cell size; thus, the water losses are not as severe as would be predicted. In the early phase, the entry of sodium and chloride into brain

cells greatly mitigates the loss of water that would otherwise occur from ideal osmotic behavior. After 7 days of hypernatremia, brain water returns to control levels because brain osmolality remains elevated. At this time, newly generated idiogenic osmoles account for as much as 60% of the increase in intracellular osmolality. Some of these idiogenic osmoles result from an increase in intracellular amino acids, particularly taurine. In addition, accumulation of osmolytes such as urea, glutamine, glycerophosphorylcholine, and myoinositol has been documented in hypernatremic rats (62).

Prevention of Hypernatremia

Because hypernatremia occurs in predictable clinical settings, early recognition may allow prevention or decreased severity of injury. Elderly persons, hospitalized patients receiving hypertonic infusions, those suffering increased insensible losses or undergoing osmotic losses, diabetic patients, and patients with previous symptoms of polydipsia and polyuria should invoke a high index of suspicion when displaying neurologic alterations.

Compared with younger individuals, geriatric patients have impaired thirst responses, decreased urinary concentrating ability, and lower baseline levels of total body water. As a result, elderly patients are the group most likely to develop severe hypernatremia in the outpatient setting, and hypernatremia in the elderly accounts for 1% to 2% of all hospital admissions. The most common scenario is that of a debilitated patient with a febrile illness. Increased insensible losses are not compensated because of impaired access to solute-free water. Recognition of mental status changes in settings of increased insensible losses should prompt close attention to the serum sodium and increased administration of solute-free water.

Table 1–5 Signs and Symptoms of Hypernatremia

Depression of sensorium
Irritability
Seizures (unusual in adults)
Focal neurologic deficits
Muscle spasticity (unusual in adults)
Signs of volume depletion (variable)
Fever

Nausea or vomiting
Labored respiration
Intense thirst

From Lanese D, Teitelbaum I. Hypernatremia. In: Jacobson HR, Striker GE, Klahr S, eds. *The Principles and Practice of Nephrology*. 2nd ed. St Louis: Mosby; 1995:896, with permission.

Hospitalized patients also are susceptible to the development of hypernatremia. Compared with outpatients, individuals developing hypernatremia during a hospital admission are more likely to be younger and to have an iatrogenic etiology (62). Inpatients with high insensible losses (e.g., patients on mechanical ventilators) develop hypernatremia because of restricted access to water and inadequate fluid prescriptions. Hypertonic fluid administration (e.g., sodium bicarbonate) and osmotic diuretics including mannitol and urea also may result in hypertonicity. Hyperosmolar tube feedings may induce diarrhea and gastrointestinal water losses, and the large daily osmolar load may lead to increased electrolyte-free water losses. Palevsky and associates (63) noted that despite frequent serum sodium measurements, treatment of hypernatremia was often delayed. Fifty percent of patients with serum sodium greater than 150 mM/L did not receive hypotonic fluid within 24 hours of becoming hypernatremic, and only 36% were corrected within 72 hours. The development of hypernatremia in the intensive care setting is associated with prolonged hospitalization and increased mortality (59,60,63,64). This is clearly, in most cases, a preventable complication.

Therapy of Hypernatremia

The primary goal in the treatment of hypernatremia is the restoration of serum tonicity. The specific approach depends on the patient's ECF volume (Fig. 1-11). The following principles are useful (65).

1. Isotonic sodium chloride should be given until systemic hemodynamics are stabilized when the patient has low total body sodium, as evidenced by circulatory manifestations (e.g., orthostatic hypotension). Thereafter, the hypernatremia can be treated with 0.45% sodium chloride or 5% dextrose.
2. When the patient is hypervolemic and hypernatremic, the removal of excess sodium is the goal, which can be achieved either by

administration of diuretics along with 5% dextrose or, if renal function is impaired, by dialysis.

3. The euvolemic hypernatremic patient who has sustained pure water losses requires water replacement as a 5% dextrose infusion. The water deficit in this setting can be calculated on the basis of the serum sodium concentration and on the assumption that 60% of the body's weight is water; the water deficit can be expressed by the equation:

$$\text{Water deficit} = 0.6 \times \text{body weight (kg)} \times (P_{NA}/140 - 1) \quad (1.5)$$

Thus, in a patient who weighs 75 kg and presents with a serum sodium of 154 mEq/L, the water deficit can be calculated as

$$\begin{aligned} &0.6 \times 75 \times (154/140 - 1) \\ &45 \times (1.1 - 1) \\ &45 \times 0.1 = 4.5 \text{ L} \\ &154/140 \times 45 \text{ L} = 49.5 \text{ L} \\ &49.5 - 45.0 = 4.5 \text{ L} \end{aligned} \quad (1.6)$$

This represents the water deficit and is the net positive water balance necessary to correct the hypernatremia. In addition, ongoing losses of free water require replacement as well to achieve normonatremia.

The general guidelines for the treatment of symptomatic hypernatremia are listed in Table 1-6 (65). The rapidity with which the correction should be made has been a matter of some controversy, primarily as concerns the pediatric population (66). In children, two studies suggest a correction rate of less than 0.5 mEq/L/h because no seizures occurred in treated children, whereas 20% of those treated more rapidly had seizures (66,67). Most feel that even in adults, correction should be achieved in more than 48 hours and at a rate not greater than 2 mEq/hour. It is likely that the described cerebral adjustments to hypernatremia, whereby brain water content is corrected and new solutes are generated, increases the risk of seizures during the correction phase. As extracellular osmolality is rapidly decreased, an osmotic gradient may develop between brain and plasma. This would result in net movement of water into the brain, causing cerebral edema. A slower rate of correction probably can prevent this sequence of events by allowing more time for the generated idiogenic osmoles to leave the brain.

Table 1–6 General Guidelines for the Treatment of Symptomatic Hypernatremia

Correct at rate of 2 mEq/L/h
Replace half calculated water deficit over first 12–24 h
Replace remaining deficit over the next 24 h
Perform serial neurologic examinations; prescribed rate of correction can be decreased with improvement in symptoms
Perform measurements of serum and urine electrolytes every 1–2 h

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In patients with essential hypernatremia and the elderly with hypodipsia, 1 to 2 L of water per day may need to be administered as a prescription. Chlorpropamide itself augments thirst, and its use in conjunction with desmopressin (DDAVP) in patients with adipsia has been proposed.

Clinical Disorders of Renal Diluting Capacity—Hyponatremic States

Although the disorders of renal concentrating capacity described in this chapter may be associated with water depletion and hypernatremia, disorders of renal diluting capacity most frequently present with hyponatremia. Sodium and its accompanying anions account for nearly all the osmotic activity of plasma (68).

$$\text{Calculated serum osmolality} = [2\text{Na}^+ + \text{blood urea nitrogen (mg/dL)}/2.8 + \text{glucose (g/dL)}/18] \quad (1.7)$$

An increase in serum sodium always reflects hyperosmolality. Hyponatremia usually is associated with hypoosmolality, and the most common setting in which the serum sodium does not reflect serum osmolality occurs when there is an additional osmolyte such as ethanol, methanol, or ethylene glycol in the ECF. An osmolar gap is said to be present when the preceding calculated serum osmolality is more than 10 mOsm lower than the osmolality directly measured by the osmometer. It must be also noted that the nature of the solute determines whether there is

an increase only in measured osmolality, but not effective osmolality and whether the serum sodium concentration is altered (Table 1-7). Solutes that are permeable across cell membranes such as urea, methanol, ethanol, and ethylene glycol increased measured, but not effective, osmolality. Thus, they do not cause water movement from cells; therefore, hypertonicity occurs without causing cellular dehydration. There is thus no alteration in serum sodium concentration. A high blood urea nitrogen and ethanol intoxication are the most common settings in which this occurs. In contrast, glucose, in the insulinopenic state, is not permeable and establishes an effective osmotic gradient for water to leave the cell and move into the ECF compartment. This process lowers the serum sodium concentration, and hyponatremia can coexist with a normal or even elevated tonicity. Conceptually, this can be viewed as "translocational" hyponatremia, as an alteration in the plasma sodium concentration level does not reflect a change in total body water but rather reflects water movement from the intracellular to the extracellular space. This effect of hyperglycemia must be considered in the interpretation of the serum sodium, and an appropriate correction must be made. The decrease in plasma sodium is approximately 1.6 mEq/L for every 100 mg/dL increase in plasma glucose, but this calculation may somewhat underestimate the decrement in serum sodium (69). The serum sodium concentration returns to normal without specific intervention as the plasma glucose is lowered. Similar decrements in serum sodium concentration following the infusion of other osmotically active substance such as mannitol, or the absorption of glycine during transurethral prostate resection or hysterectomies.

Table 1–7 Relationship between Serum Tonicity and Sodium Concentration in the Presence of Other Substances

Condition of Substance	Serum Tonicity	Serum Sodium
Hyperglycemia	↑	↓
Mannitol, maltose, glycine	↑	↓
Anzotemia (high blood urea)	↑	↔
Ingestion of ethanol, methanol, ethylene	↑	↔

glycol		
Elevated serum lipid and/or protein	↔	↓[a]

[a]As measured by flame photometer.
From Berl T. Robertson GL. Pathology of water metabolism. In: Brenner BM, Rector FC Jr, eds. *The Kidney*. 6th ed. Philadelphia: WB Saunders, 2000:894, with permission.

Pseudohyponatremia occurs when the solid phase of plasma (normally 6%–8%) is markedly increased by large increments in serum lipids or protein, because the flame photometer measures sodium concentration of the entire plasma and not just the liquid phase concentrations. The use of a direct ion selective electrode, measuring only the sodium concentration of the liquid phase, eliminates this problem. Only a direct potentiometry measurement (undiluted sample) gives an accurate determination in this setting. The impact of specific increments in lipids and protein on measured serum sodium has been experimentally quantified (70), leading the authors to suggest the following corrective formula:

$$\text{Plasma water content (\%)} = 99.1 - (0.1 \times \text{L}) - (0.07\text{X} \times \text{P}) \quad (1.8)$$

where L refers to the lipid and P to the protein concentrations in g/L. For example, if the formula reveals that plasma water is 90% of the plasma sample rather than the normal 93% (which yields a serum sodium of 140 mmol/L as 150 × 0.93 = 140), the concentration of measured sodium would be expected to decrease to 135 mmol/L (150 × 0.90).

Hyponatremia is the most common electrolyte disorder seen in clinical practice. Hyponatremia may be associated with decreased, increased, or near-normal amounts of total body sodium (5,71). This section attempts to discuss and categorize the disorders of renal diluting capacity and associated hyponatremia in relationship to total body sodium and ECF volume status. In each instance, the pathogenetic mechanisms that may be involved are considered.

Disorders of diluting capacity, depicted in Figure 1-1A, are caused by: (a) the continued secretion of AVP in spite of the presence of serum hypoosmolality, which does not allow the collecting duct to remain water impermeable; or (b) intrarenal factors, such as a decrease in GFR or proximal tubular fluid and sodium reabsorption, or both, which diminish the delivery of fluid to the distal diluting segments of the nephron. A

defect in sodium chloride transport out of the water-impermeable portions of the nephron, including the cortical and medullary ascending limb of the loop of Henle, and particularly in the distal convoluted tubule is another intrarenal factor that impairs the nephron's capacity to dilute tubular fluid and urine.

In Figure 1-14 are summarized the diagnostic and therapeutic approaches to hyponatremia discussed in this chapter. After the patient's hyponatremia is determined to reflect a truly hypotonic state, a thorough history and physical examination are essential in order to assess the volume status of the patient with hyponatremia. The diagnostic possibilities are narrowed once the patient is placed in one of three categories (edematous states, hypovolemic states, or neither). Examination of the urinary sodium concentration provides supportive evidence for the diagnosis. This diagnostic approach to hyponatremia also makes the appropriate therapy easy to define (5,65).

HYPONATREMIA WITH LOW TOTAL BODY SODIUM

In addition to hypothalamic osmoreceptors, another "physiologic" control of AVP release is the patient's volume status, sensed by arterial baroreceptors (Fig. 1-7). When the osmoreceptor and arterial baroreceptors provide opposing stimuli for AVP release, the effect of the baroreceptors usually predominates. Thus, in the presence of hypovolemia, AVP release is stimulated and water is retained, even at the expense of hyposmolality. The glossopharyngeal and vagal afferent pathways from the aortic arch and carotid sinus are normally inhibited by nonosmotic AVP stimulation. With a decrease in arterial pressure, these baroreceptors are unloaded and AVP release is stimulated independent of osmolality. Receptors in the left atrium that are also present may modulate vagal afferent tone and AVP release. In the presence of volume depletion, a fall in pressure at the level of both the arterial baroreceptors and the left atrium may inhibit afferent neural tone, an effect known to stimulate AVP release. Thus, the baroreceptor-stimulated secretion of vasopressin, coupled with high water intake (either oral or parenteral), culminates in hyponatremia.

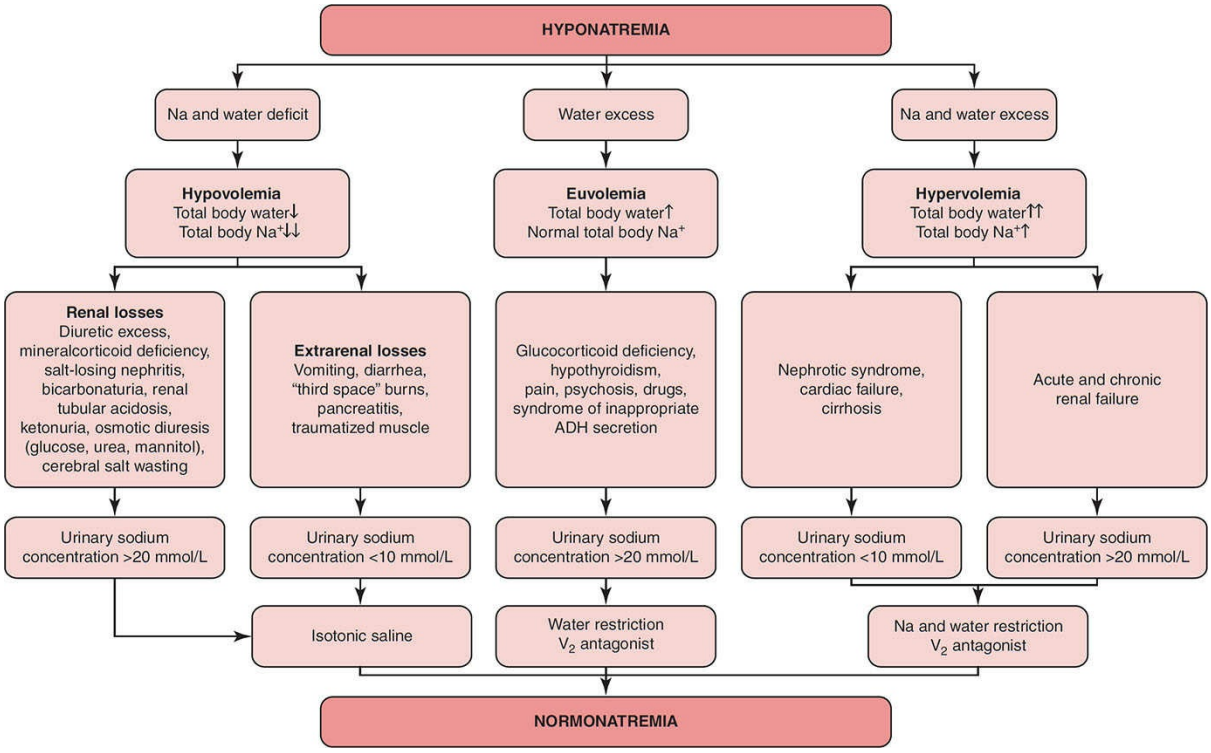

Figure 1–14 Diagnostic and therapeutic approach to the hyponatremic patient. (From Berl T, Kumar S. Disorders of water balance in comprehensive clinical nephrology. In: Johnson RJ, Feehally J, eds. *Comprehensive Clinical Nephrology*. St Louis: CV Mosby; 2000:3–9.7, with permission.)

The presence of hypovolemia, as judged by weight loss, orthostatic hypotension and tachycardia, and decreased central venous pressure, in association with hyponatremia, raises the question as to the source of the fluid and electrolyte losses. There are primarily two main sources for such losses, the gastrointestinal track and the kidneys.

Gastrointestinal and "Third" Space Losses

In the presence of gastrointestinal losses (through either vomiting or diarrhea), the kidney responds by conserving sodium chloride. A similar pattern is observed with sequestration of fluids into third spaces, as in the peritoneal cavity in peritonitis and pancreatitis, or the bowel lumen with ileus and burns. In these entities, the urinary sodium concentration should be less than 10 mEq/L if renal function is normal. The urine osmolality also should be in the hyperosmolar range. Vomiting and metabolic alkalosis, in which bicarbonaturia is present, are exceptions. The urinary bicarbonate anion obligates cations and consequently may be associated with a urinary sodium concentration greater than 20 mEq/L. However, the urinary chloride concentration is less than 10 mEq/L.

Renal Losses

Diuretics

Excessive use of diuretics is one of the most common situations in which hyponatremia is associated with hypovolemia (72). Advanced age in underweight women appears to be an important risk factor. Hyponatremia occurs almost exclusively with thiazide rather than loop diuretics, because the former do not impair urinary concentration but the latter do. Hyponatremia supervenes within 14 days of initiating diuretic therapy. Diuretics cause hyponatremia by at least three mechanisms: (a) volume depletion, which results in impaired water excretion by both an enhanced AVP release and decreased fluid delivery to the diluting segment; (b) a direct effect of diuretics on the diluting segment; and (c) potassium depletion. The mechanism whereby potassium depletion itself leads to hyponatremia is not entirely understood. However, it appears that it can occur independent of the sodium depletion that frequently accompanies diuretic use. An effect of hypokalemia to stimulate thirst and therefore increase water intake could aggravate any of the aforementioned mechanisms. It must be noted that the concomitant administration of potassium-sparing diuretics does not prevent the development of hyponatremia. Although the diagnosis of diuretic-induced hyponatremia frequently is obvious, surreptitious diuretic abuse is being recognized increasingly and should be considered in patients whose other electrolyte abnormalities such as hypokalemic metabolic alkalosis, and high urinary chloride excretions suggest this possibility.

Salt-Losing Nephritis

Salt-losing nephritis is another condition with which hyponatremia and hypovolemia may be associated. In most circumstances, salt-losing nephritis is associated with advanced chronic renal disease (GFR <20 mL/minute). Nevertheless, salt-losing nephritis may occur with less severe renal impairment with certain diseases, such as medullary cystic disease, polycystic disease, analgesic nephropathy, obstructive nephropathy, and chronic pyelonephritis.

Certain patients with renal tubular acidosis, particularly those with the proximal type II variety, may exhibit sodium and potassium wastage despite only modest decreases in GFR. These patients have prominent bicarbonaturia because of a defect in the proximal tubule affecting the reclamation of bicarbonate (Chapter 3). As mentioned, bicarbonate is a

relatively impermeable anion that obligates the renal excretion of cations, including primarily sodium and potassium. In this setting of renal tubular acidosis, the bicarbonaturia obligates the excretion of sodium, so that a minimal urinary sodium concentration is not achieved despite the presence of hypovolemia.

Mineralocorticoid Deficiency

A finding of hyponatremia and ECF volume depletion also suggests the possibility of primary adrenal insufficiency, particularly in the presence of a urinary sodium concentration higher than minimal, that is, greater than 20 mEq/L. Because of the mineralocorticoid deficiency, a diminished urinary excretion of potassium and hyperkalemia is an indication that primary adrenal insufficiency is the cause of the hyponatremia. It is worthy of mention that urinary sodium concentration may become minimal when hypopituitarism is associated with hyponatremia because the renin–angiotensin–aldosterone pathway is intact.

The mechanisms whereby adrenal insufficiency impairs renal water excretion have been the subject of considerable debate, and the effects of mineralocorticoid and glucocorticoid deficiency need to be considered separately (39). Studies in experimental animals suggest that mineralocorticoid deficiency and an associated negative sodium balance are at least partially responsible for impaired water excretion and hyponatremia as AVP secretion mediates this effect of sodium depletion on renal water excretion. AQP_2 and AQP_3 expressions are increased but the Na/K/2Cl transporter in the outer medulla is decreased in mineralocorticoid-deficient animals (73). ECF volume depletion with increased AVP and diminished renal hemodynamics mediate the effect of mineralocorticoid deficiency to cause hyponatremia.

Osmotic Diuresis

An osmotic diuresis can lead to urinary losses of sodium and water, volume depletion, and hyponatremia in the face of either oral or parenteral water intake. The uncontrolled diabetic patient with glucosuria, and the patient with a urea diuresis after relief of urinary tract obstruction or undergoing a mannitol diuresis are examples of such causes of hyponatremia. The urinary sodium concentration generally is greater than 20 mEq/L because the osmotic diuresis obligates cation excretion in spite of concomitant volume depletion. In patients with diabetes, the urinary

sodium wasting caused by the glycosuria can be accentuated by ketonuria, as hydroxybutyrate and acetoacetate, which also obligate urinary electrolyte losses. Ketonuria may contribute to the renal sodium wasting and hyponatremia seen in starvation and alcoholic ketoacidosis.

Cerebral Salt Wasting

Urinary sodium also exceeds 20 mEq/L, despite hypovolemia in cerebral salt wasting, a syndrome primarily described in patients with subarachnoid bleeds. The mechanism is not fully understood and the hyponatremia cannot be readily differentiated from the SIADH secretion, since the secretion of the hormone is critical to the development of the hyponatremia and the presence of hypovolemia is not always fully supported (74,75).

HYPONATREMIA WITH INCREASED TOTAL BODY SODIUM

Hyponatremia is frequently observed in edematous disorders. In this setting, both total body sodium and total body water are increased, but total body water is increased to a greater extent (Fig. 1-14). Because diuretics are used frequently in edematous disorders, hyponatremia presents a diagnostic dilemma as to whether the impairment in water excretion is due to the diuretic therapy. This is because the edematous disorders—including cirrhosis, cardiac failure, and nephrotic syndrome—may impair renal water excretion and be associated with hyponatremia in the absence of diuretic use.

Congestive Heart Failure

The common association between congestive heart failure and sodium and water retention is well established (39,76,77). The hyponatremia, a powerful prognostic factor in patients with heart failure, may be mediated by either a decreased delivery of tubular fluid to the distal nephron or an increased release of vasopressin, or both.

The decrement in “effective arterial blood volume” with the decrease in arterial filling, secondary to a low cardiac output that is sensed by aortic and carotid sinus baroreceptors, causes nonosmotic stimulation of vasopressin release (76). This stimulation must supersede the inhibition in AVP release that usually accompanies acute distention of the left atrium

that occurs with cardiac failure. Experiments using an AVP antagonist in a model of low cardiac output also point to an important role for the hormone in abnormal urinary dilution.

Following the development of a radioimmunoassay for vasopressin, several studies demonstrated elevated plasma AVP levels in patients with heart failure. It is most likely that nonosmotic pathways, whose activation is suggested by the increase in sympathetic activity seen in congestive heart failure, are the mediator of the hormone's release. Thus, an improvement in cardiac function with afterload reduction decreased AVP levels and improved water excretion in such patients. A decrease in plasma AVP levels also accompanied improvement in cardiac function by hemofiltration. It is of note that the V_2 antagonist decreased urinary osmolality, increased solute-free water excretion, and increased serum sodium concentration in heart failure patients (78–80). AQP_2 expression is also increased in experimental heart failure (81) (Fig. 1-13).

Hepatic Failure

Patients with advanced cirrhosis and ascites frequently present with hyponatremia as a consequence of their inability to excrete a water load (39). Early in cirrhosis, the increase in portal pressure increases splanchnic blood flow leading to a decrease in vascular decrease in systemic vascular resistance. This in turn leads to arterial underfilling and activation of neurohumoral pathways including norepinephrine, the renin–angiotensin system, and the nonosmotic release of vasopressin. In fact, vasopressin levels have been found to be elevated in patients with cirrhosis and ascites. A predominant role for AVP secretion in the pathogenesis of the disorder has been demonstrated in experimental animals (82) and in humans (83). However, a vasopressin-independent intrarenal mechanism may contribute to the defect in water excretion as well. Nitric oxide (NO) may be an important mediator of the vasodilatation in this disorder (84), as inhibition of NO corrects the arterial hyporesponsiveness to vasodilators (85) and the abnormal water excretion in cirrhotic rats (84). As in heart failure, AQP_2 expression is increased in cirrhotic rats (86).

Nephrotic Syndrome

The incidence of hyponatremia in the nephrotic syndrome is lower than in either congestive heart failure or cirrhosis. An elevated level of plasma

vasopressin also has been shown to occur in patients with the nephrotic syndrome. In view of the alterations in Starling forces that accompany hypoalbuminemia and allow transudation of salt and water across capillary membranes into the interstitial space, many patients with the nephrotic syndrome have been considered to have intravascular volume contraction leading to nonosmotic release of vasopressin. Although this mechanism is most likely operant in minimal change disease and those with normal GFR, it may be less applicable to other patients with the nephrotic syndrome. Some of these patients had increased plasma volumes with suppressed plasma renin activity and aldosterone levels. Such patients usually have decrements in GFR and a primary renal sodium-retaining disorder. In contrast to the increased AQP_2 found in the described sodium-retaining disorders, the expression of the water channels is decreased in models of the nephrotic syndrome (87). The animals did not have hyponatremia and most likely had volume expansion to explain the discrepancy.

Advanced Chronic Renal Failure

The combination of hyponatremia and edema also may occur in patients with advanced renal failure, whether resulting from acute or chronic renal disease (48,88). As was the case in heart failure and cirrhosis, the presence of hyponatremia also is associated with increased mortality in patients with chronic kidney disease (89). Unlike subjects with edematous disorders, these patients do not have a minimal urinary sodium concentration because of the accompanying tubular dysfunction. The chronically diseased kidney also may exhibit a profound increase in fractional sodium excretion in an effort to maintain sodium balance despite its reduced number of functioning nephrons. Generally, edema develops because larger amounts of sodium are ingested than can be excreted by the diseased kidneys, which are filtering only a fraction of the amount of sodium filtered by normal kidneys. For example, at a GFR of 100 mL/minute (144 L/day) and a plasma sodium concentration of 140 mEq/L, the daily filtered load of sodium (GFR × plasma sodium concentration) is 20,160 mEq. With a reduction in GFR to 5 mL/minute, the daily amount of sodium filtered is only 1,008 mEq. The fractional excretion of sodium necessary to maintain sodium balance is much greater in the latter circumstance.

The narrow range of water handling by the diseased kidney is probably also caused in large part by the smaller volumes of fluid that are filtered daily by the diseased kidney. At a GFR of 5 mL/minute, only 7.2 L of

filtrate is formed daily, and perhaps 30%, or 2.2 L, of this filtered fluid will reach the diluting segment of the nephron. Thus, even with total suppression of AVP and water impermeability of the collecting duct, a maximum of 2.2 L of solute-free water could be excreted daily. If the daily water intake exceeds this volume, plus insensible losses, then a positive water balance and hyponatremia occur. Thus, in advanced chronic renal failure, the volume of fluid filtered and delivered to the diluting segment is of paramount importance to the renal capacity to excrete water. Although most patients with advanced renal failure (GFR <10 mL/minute) have little capacity to concentrate urine, some capacity to dilute urine may be preserved (48). However, the capacity to maintain water balance is dependent not only on the ability to dilute urine but also on the quantitative capacity of the kidney to excrete C_{H2O}. With acute renal injury, the near absence of GFR explains the inability of such kidneys to respond to a water load.

HYPONATREMIA WITH NORMAL TOTAL BODY SODIUM

In Figure 1-14 are listed the causes of euvolemic hyponatremia.

Glucocorticoid Deficiency

Glucocorticoid deficiency is important in the impaired water excretion of primary and secondary adrenal insufficiency. The mechanism is distinct from the one described for mineralocorticoid deficiency as there is no negative sodium balance and hypovolemia (39). An elevation of plasma AVP levels accompanies the water excretory defect of patients, anterior pituitary insufficiency, and glucocorticoid-deficient animals (90). Vasopressin antagonists reverse the water-retaining disorder (91). AQP_2 expression is also increased in the medullary tissue of such animals (91). A direct effect of glucocorticoids in magnocellular neurons that are endowed with receptors for hormone also has been proposed (92). It seems clear, however, that ADH-independent factors are also involved in impaired water excretion with glucocorticoid deficiency. Whereas the AVP-dependent component may be observed in adrenalectomized, mineralocorticoid-replaced rats deprived of glucocorticoid hormone for 24 hours, AVP-independent impairment in water excretion occurs after 2 weeks of glucocorticoid deficiency in AVP-deficient Brattleboro rats. The AVP-independent effect is associated with impaired renal hemodynamics and decreased distal fluid delivery to the diluting segment of the nephron.

Hypothyroidism

Hyponatremia may develop in some patients with advanced hypothyroidism, even in the absence of cardiac failure but it is so unusual that some have questioned the association (93). This is most likely owing to the fact that the impaired water excretion occurs only in severe hypothyroidism. Several mechanisms have been proposed to explain this impaired diluting capacity with hypothyroidism. Studies in hypothyroid humans and rats have demonstrated elevated levels of plasma vasopressin, thus implicating AVP in the impaired water excretion associated with thyroid hormone deficiency. On the other hand, studies of osmoregulation in hypothyroid patients have found that both the threshold and the sensitivity of the vasopressin response are normal (94). Such an observation incriminates an intrarenal hemodynamic disturbance. A study in rats with severe hypothyroidism revealed an almost complete reversal of the water excretory defect when given a V_2 vasopressin receptor antagonist, suggesting a minor role for an AVP-independent mechanism (95). These rats also had elevated AQP_2 expression in the inner medulla.

Psychosis—Primary Polydipsia

Acutely psychotic patients, particularly those with schizophrenia, are at risk of developing hyponatremia (96). Polydipsia occurs in approximately 20% of psychiatric patients. The elucidation of the mechanism has been confounded, because possible pharmacologic agents, such as nicotine, thiazides, and carbamazepine, frequently are implicated (93). Psychiatric medications also cause dry mouth and stimulate thirst. However, reports of psychotic patients with water intoxication who are not taking medications also exist. The mechanism of the hyponatremia associated with psychosis thus appears to be multifactorial (97). Thirst perception is increased, there is a nonosmotic stimulus that causes AVP to be secreted at lower osmolality, and the renal response to AVP may be enhanced. Although each derangement alone would be insufficient to cause overt hyponatremia, their combination very well may cause overt hyponatremia (98). The combination of low solute intake with high water intake makes those subjects more prone to develop hyponatremia (99).

Postoperative Hyponatremia

The incidence of hospital-acquired, particularly postoperative,

hyponatremia is increased in adults (100) as well as in children (101). Most of the patients are euvolemic and have measurable plasma AVP levels (100). The hyponatremia is asymptomatic in most of the patients, but there is a subgroup of postoperative women with cerebral edema who have seizures and hypoxia with catastrophic neurologic events (102).

Pharmacologic Agents

Drugs associated with water retention are listed in Table 1-8. Drug-induced hyponatremia is becoming the most common cause of hyponatremia (72). Thiazide diuretics are the most common cause, probably followed by selective serotonin uptake inhibitors (SSRI). Hyponatremia can be mediated by vasopressin analogues such as desmopressin (brand name DDAVP [1-desamino-D-arginine vasopressin]), drugs that enhance vasopressin release, and agents potentiating the action of vasopressin. In other instances, the mechanism is unknown. The increased use of desmopressin for nocturia in the elderly and enuresis in the young has resulted in a marked increase in reported cases of hyponatremia in these subjects (103). The increasing use of intravenous immunoglobulin (IVIG) as a therapeutic modality in many disorders, cases of hyponatremia associated with its use, has been described (104). The mechanism of IVIG-associated hyponatremia is multifactorial involving pseudohyponatremia as the protein concentration increases, translocation because of the sucrose present in the solution, and true dilutional hyponatremia related to retention of water, particularly in those with associated acute kidney injury. An increasing number of antipsychotic agents have been associated with hyponatremia, and they are frequently implicated in an explanation for the water intoxication in psychotic patients. The role of the drugs in the etiology of the impaired water excretion in patients receiving the agents has not been dissociated, in most cases, from the role of the underlying psychiatric disorder for which the patient is receiving the drug. Therefore, although the clinical association between antipsychotic drugs and hyponatremia frequently is encountered, the pharmacologic agents themselves may not be the primary factors responsible for the water retention. Of particular interest is the frequent occurrence of hyponatremia in elderly subjects receiving SSRI drugs. The incidence has been reported to be as high as 22% to 28% in some studies (105). An increasing number of cases of hyponatremia have been reported after the use of the recreational drug 3,4-methylenedioxymethamphetamine (“ecstasy”) (106).

Table 1–8 Drugs Associated with Hyponatremia

Antidiuretic hormone analogs
Deamino-D-arginine vasopressin
Oxytocin
Drugs that enhance antidiuretic hormone release
Chlorpropamide
Clofibrate
Carbamazepine and oxcarbazepine
Vincristine
Nicotine
Narcotics (μ-opioid receptors)
Antipsychotics/antidepressants
Ifosfamide
Drugs that potentiate renal action of antidiuretic hormone
Chlorpropamide
Cyclophosphamide
Nonsteroidal antiinflammatory drugs
Acetaminophen
Drugs that cause hyponatremia by unknown multiple mechanisms
Haloperidol
Fluphenazine
Amitriptyline
Serotonin uptake inhibitors
"Ecstasy" (amphetamine related)
IVIG

IVIG, intravenous immunoglobulin.
From Veis JH, Berl T. Hyponatremia. In: Jacobson HR, Striker GE, Klahr S, eds. *The Principles and Practice of Nephrology.* 2nd ed. St Louis: Mosby, 1995:890, with permission.

Exercise-Induced Hyponatremia

Hyponatremia is increasingly seen in long-distance runners. A study at a marathon race found that a body mass index below 20 kg/m^2, running time exceeding 4 hours, and greatest weight gain (107) were associated with increased risk of developing hyponatremia. A study in ultramarathon runners showed elevated vasopressin despite normal or low serum sodium

(108).

Syndrome of Inappropriate Antidiuretic Hormone Secretion

Pathophysiology

The chronic administration of AVP when accompanied with an intake of water culminates in the development of hyponatremia (109). As depicted in Figure 1-15, the continued administration of AVP is accompanied by a decline in the hydroosmotic effect of the hormone as urine osmolality falls and serum sodium stabilizes, a phenomenon denoted as vasopressin escape. A downregulation of receptors, possibly by activation of an inhibitory G protein, has been suggested. This may explain the decrement in cAMP generation and the decrease in AVP-stimulated water permeability during vasopressin escape (110). This is also associated with downregulation of AQP_2 but not AQP_3 (111). Hypotonic volume expansion also is necessary to achieve vasopressin escape.

Clinical Settings

The diagnosis of SIADH is made primarily by excluding other causes of hyponatremia. A diagnosis of SIADH should be considered in the absence of hypovolemia, edematous disorders, endocrine dysfunction (including primary and secondary adrenal insufficiency and hypothyroidism), renal failure, and drugs, all of which may impair water excretion. In Table 1-9 are summarized the diagnostic criteria for the syndrome and Table 1-10 lists various diseases in which SIADH may occur. These associated diseases generally fall into three categories: malignancies, pulmonary disorders, and CNS disorders. Hyponatremia without apparent cause, so-called idiopathy, is not uncommon in elderly patients (112). SIADH is characterized by the excretion of concentrated urine in the presence of blood hypotonicity. The urinary sodium concentration generally is greater than 20 mEq/L reflecting the usual sodium-rich dietary intake. However, the urinary sodium concentration may decrease to less than 1 mEq/L if patients are placed on a low-sodium diet or become volume depleted.

Most patients with SIADH have a defect in the osmoregulation of vasopressin. Robertson and his colleagues (113) reported plasma vasopressin measurements in 106 patients who fulfilled the clinical criteria for the diagnosis of SIADH before the correction of their hyponatremia. In the vast majority, plasma vasopressin concentration was inadequately

suppressed relative to the hyposmolality present. Interestingly, plasma vasopressin was between 1 and 10 pg/mL in most patients, the same range as in normonatremic hydrated, healthy adults, which indicates that nonosmotic AVP secretion can best be demonstrated under hypotonic conditions. As seen in Figure 1-16, four patterns are evident. Type A is associated with large and erratic fluctuations in plasma vasopressin, which bore no relationship to the rise in plasma osmolality. This pattern was found in 6 of 25 patients studied who had acute respiratory failure, bronchogenic carcinoma, pulmonary tuberculosis, schizophrenia, and rheumatoid arthritis. This pattern indicates that the secretion of vasopressin either had been totally divorced from osmoreceptor control or was responding to some periodic nonosmotic stimulus. A completely different type of osmoregulatory defect is exemplified by type B response, as seen in Figure 1-16. The infusion of hypertonic saline resulted in a prompt and progressive rise in plasma osmolality. Regression analysis showed the precision and sensitivity of this response to be essentially the same as in healthy subjects but the intercept or threshold value at 253 mOsm/kg to be well below the normal range. This pattern, which reflects the “resetting of the osmoreceptor,” was found in 9 of the 25 patients who had the diagnosis of bronchogenic carcinoma, cerebrovascular disease, tuberculous meningitis, acute respiratory disease, and carcinoma of the pharynx. Another patient with hyponatremia and acute idiopathic polyneuritis had an identical pattern to a hypertonic saline infusion and was determined to have resetting of the osmoreceptor. This and other patients with a reset osmostat have been able to dilute their urine maximally and sustain a urine flow sufficient to prevent a further increase in body water. Thus, an abnormality in AVP regulation can exist in spite of the ability to dilute the urine maximally and excrete a water load, a situation reminiscent of the hypotonicity seen in pregnancy. This was demonstrated however, with an acute 20 mL/kg water load which lowers plasma osmolality much more rapidly than seen clinically.

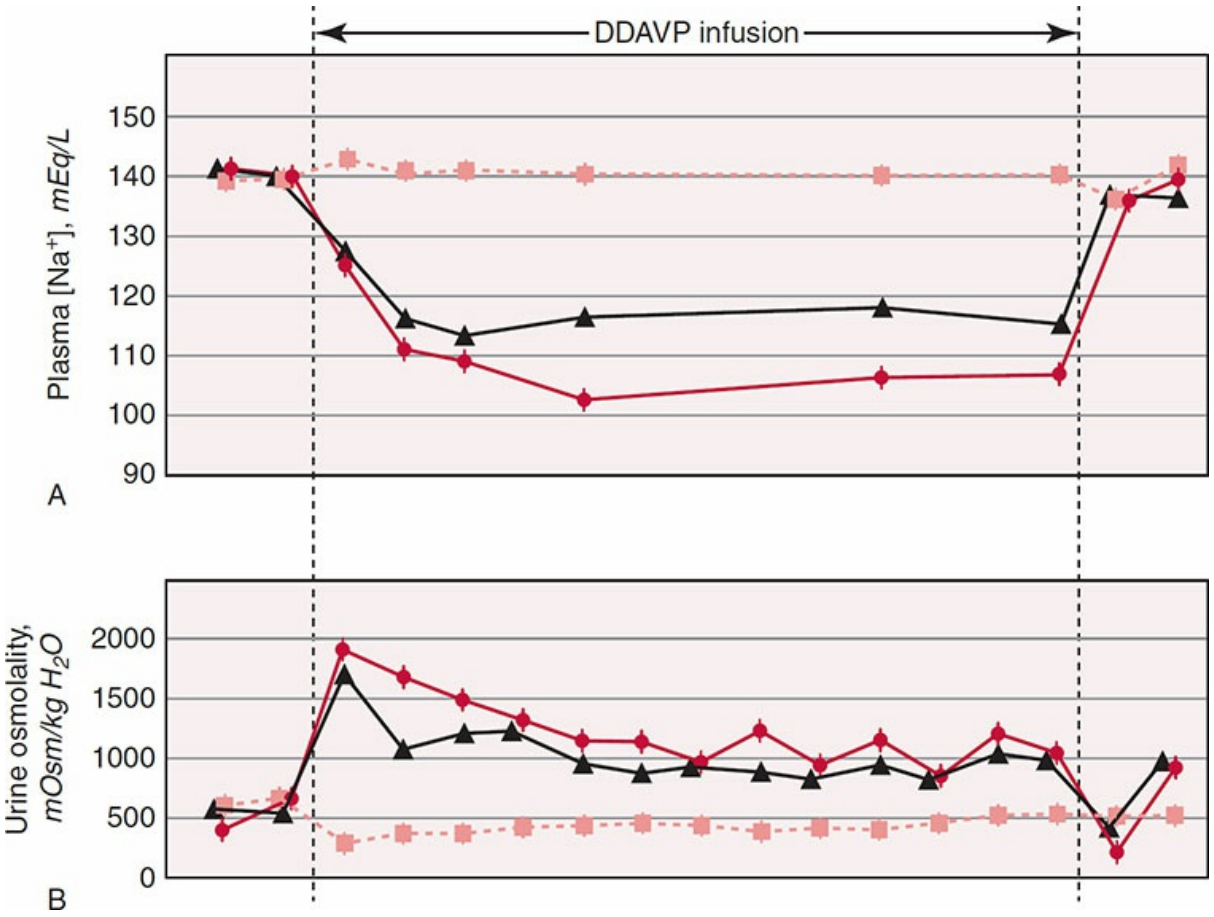

Figure 1–15 Effects of Pitressin and water administration. Note that urine flow increases, urine osmolality decreases, and serum sodium stabilizes. DDAVP, desmopressin acetate. (Reprinted from Verbalis JG, Drutarosky M. Adaptation to chronic hypoosmolality in rats. *Kidney Int*. 1988;34 (3):351–360, with permission from Elsevier.)

Table 1–9 Diagnostic Criteria for the Syndrome of Inappropriate ADH Release

Essential Diagnostic Criteria	Supplemental Criteria
Decreased extracellular fluid effective osmolality (<mOsm/kg H_2O)	Abnormal water-load test (inability to excrete at least 90% of a 20-mL/kg water load in 4 h and/or failure to dilute urine osmolality to <100 mOsm/kg)
Inappropriate urinary concentration (>100 mOsm/kg H_2O)	Plasma vasopressin level inappropriately elevated relative to plasma osmolality
Clinical euvolemia	No significant correction of plasma Na^+ level with volume expansion, but
Elevated urinary Na^+ concentration under conditions of normal salt and water intake	
Absence of adrenal, thyroid, pituitary,	

or renal insufficiency or diuretic use	improvement after fluid restriction

(Reprinted from Parikh C, Berl T. Disorders of water metabolism. In: London MI, ed. *Comprehensive Clinical Nephrology*. 4th ed. 2010:100–117, with permission from Elsevier.)

Table 1–10 Disorders Associated with the Syndrome of Inappropriate Antidiuretic Hormone Secretion

Carcinomas	Pulmonary Disorders	Central Nervous System Disorders	Other
Bronchogenic carcinoma Carcinoma of the duodenum Carcinoma of the pancreas Thymoma Carcinoma of the stomach Lymphoma Ewing sarcoma Carcinoma of the bladder Prostatic carcinoma Oropharyngeal tumor	Viral pneumonia Bacterial pneumonia Pulmonary abscess Tuberculosis Aspergillosis Positive pressure breathing Asthma Pneumothorax Mesothelioma Cystic fibrosis	Encephalitis (viral or bacterial) Meningitis (viral, bacterial, tuberculous, and fungal) Carcinoma of the ureter Head trauma Brain abscess Guillain–Barré syndrome Acute intermittent porphyria Subarachnoid hemorrhage or subdural hematoma Cerebellar and cerebral atrophy Cavernous sinus thrombosis Neonatal hypoxia Shy–Drager syndrome Rocky Mountain	Acquired immunodeficiency syndrome Prolonged exercise Idiopathic (in elderly)

	spotted fever Delirium tremens Cerebrovascular accident (cerebral thrombosis or hemorrhage) Acute psychosis Peripheral neuropathy Multiple sclerosis

From Levi M, Berl T. Water metabolism. In: Gonick HC, ed. *Current Nephrology*. Vol 5. Chicago: Year Book Medical Publishers; 1982:45, with permission.

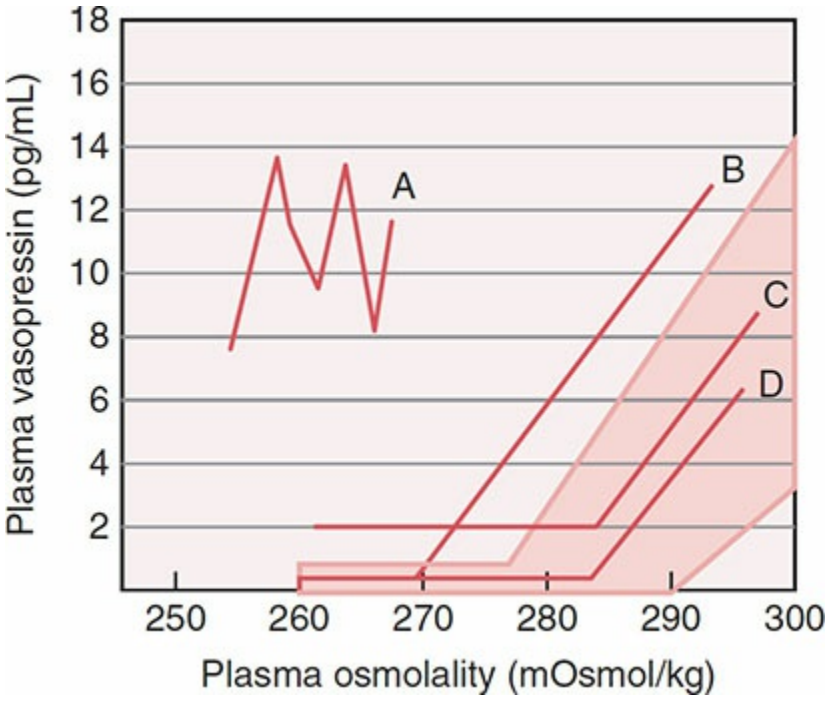

Figure 1–16 Plasma vasopressin as a function of plasma osmolality during the infusion of hypertonic saline in four groups of patients with clinical syndrome of inappropriate antidiuretic hormone (SIADH). The shaded area indicates the range of normal value. (From Verbalis J, Berl T. Disorders of water balance. In: *The Kidney*. 8th ed. Philadelphia: Saunders Elsevier; 2008:493, with permission.)

As seen in Figure 1-16, in the type C response, plasma vasopressin was elevated initially but did not change during the infusion of hypertonic saline until plasma osmolality reached the normal range. At that point, plasma AVP began to rise appropriately, indicating a normally functioning osmoreceptor mechanism. This response was found in 8 of the 25 patients with the diagnosis of CNS disease, bronchogenic carcinoma, carcinoma of

the pharynx, pulmonary tuberculosis, and schizophrenia. Its pathogenesis is unknown, but it was speculated that this variety of SIADH may result from a constant, nonsuppressible leak of AVP despite otherwise normal osmoregulatory function. Unlike type II, or the resetting type of defect, it results in impaired urinary dilution and water excretion.

As seen in Figure 1-16, in the type D response, the osmoregulation of vasopressin appeared to be completely normal despite the marked inability to excrete a water load. The plasma AVP was appropriately suppressed under hypotonic conditions and did not rise until plasma osmolality reached the normal threshold level. When this procedure was reversed by water loading, plasma osmolality and plasma AVP again fell normally, but urinary dilution did not occur and the water load was not excreted. This was seen in 10% of the studied patients. Thus, these patients had no detectable AVP when they were hyponatremic. It is possible that at least some of these patients have a recently described defect in the V_2 receptor that renders it constitutively active (114). This disorder has been termed nephrogenic syndrome of inappropriate antidiuresis. The four patterns of defective osmoregulation described above have more recently been confirmed employing a copeptin assay as a marker for vasopressin (115). However, none of the six patients displaying pattern D (no measurable vasopressin levels) were found to have a gain-of-function mutation.

SIGNS AND SYMPTOMS OF HYPONATREMIA

The most common signs and symptoms of hyponatremia are listed in Table 1-11. Most patients with hyponatremia are asymptomatic. Although gastrointestinal complaints occur early, the majority of the manifestations of hyponatremia are of a neuropsychiatric nature and include lethargy, psychosis, seizures, and coma. These symptoms reflect the brain edema that accompanies the osmotic water shift. This is denoted as hyponatremic encephalopathy (71,116). The water movement may be mediated by AQP_4, since animals with knockout of this water channel seem protected from brain swelling (117) and those that overexpress it are prone to brain edema (118). In the most severe form, hyponatremia encephalopathy can cause brainstem compression leading to respiratory failure and pulmonary edema leading to hypoxia (119). Elderly patients and young children with hyponatremia are most likely to become symptomatic. Also, it has become apparent that neurologic complications occur more frequently in menstruating women. In a case–control study, despite approximately equal incidence of postoperative hyponatremia in males and females, 97% of

those with permanent brain damage were women and 75% of them were menstruant (120). The severity of symptoms is dependent on the rate at which serum sodium concentration is lowered as well. There is considerable disagreement as to the mortality of acute hyponatremia. This has been reported to be as high as 50% and as low as 3%. There is general agreement that the mortality of chronic hyponatremia in hospitalized patients is 10% to 27%. However, the deaths are generally caused by the underlying disorder rather than the hyponatremia per se. The mortality is lower with chronic hyponatremia because brain volume regulatory responses protect against cerebral edema over time. Studies in rats demonstrate a loss of both electrolyte and organic osmolytes after the onset of hyponatremia. Although some of the osmolyte losses occur within 24 hours, the loss of such solutes becomes more marked in subsequent days and account for almost complete restoration of cerebral water volume. The electrolytes and other osmolytes lost in the adaptation to hyponatremia are shown in Figure 1-17. The rate at which the brain restores the lost electrolytes and osmolytes when hyponatremia is corrected is of great pathophysiologic importance. Sodium and chloride recover quickly and even overshoot. However, the accumulation of osmolytes is considerably delayed, perhaps because of the reported downregulation by hypotonicity of amino acid transporters, including some osmolytes, thus impairing their restoration in the brain (121). This process is likely to account for the more marked cerebral dehydration that accompanies the rapid correction in previously adapted animals with chronic hyponatremia as well as alteration in the blood–brain barrier that may underlie the development of demyelinating lesions (122).

THERAPY OF HYPONATREMIA

The treatment of hyponatremia has been the subject of considerable interest and controversy (65,122–124). The strategy is dictated by the underlying cause, clinical severity, and timing of hyponatremia development. Unfortunately, the identification of the underlying pathology is not evident immediately. Management should be based on whether the patient is or is not symptomatic and whether the disorder is acute or chronic.

Table 1–11 Symptoms and Signs That May Be Associated with Hyponatremia

Symptoms	Signs
Lethargy, apathy	Abnormal sensorium
Disorientation	Depressed deep tendon reflexes
Muscle cramps	Cheyne–Stokes respiration
Anorexia, nausea	Hypothermia
Agitation	Pathologic reflexes
—	Pseudobulbar palsy
—	Seizures

(Reprinted from Berl T, Anderson RJ, McDonald KM, et al. Clinical disorders of water metabolism. *Kidney Int.* 1976;10(1):117–132; with permission from Elsevier.)

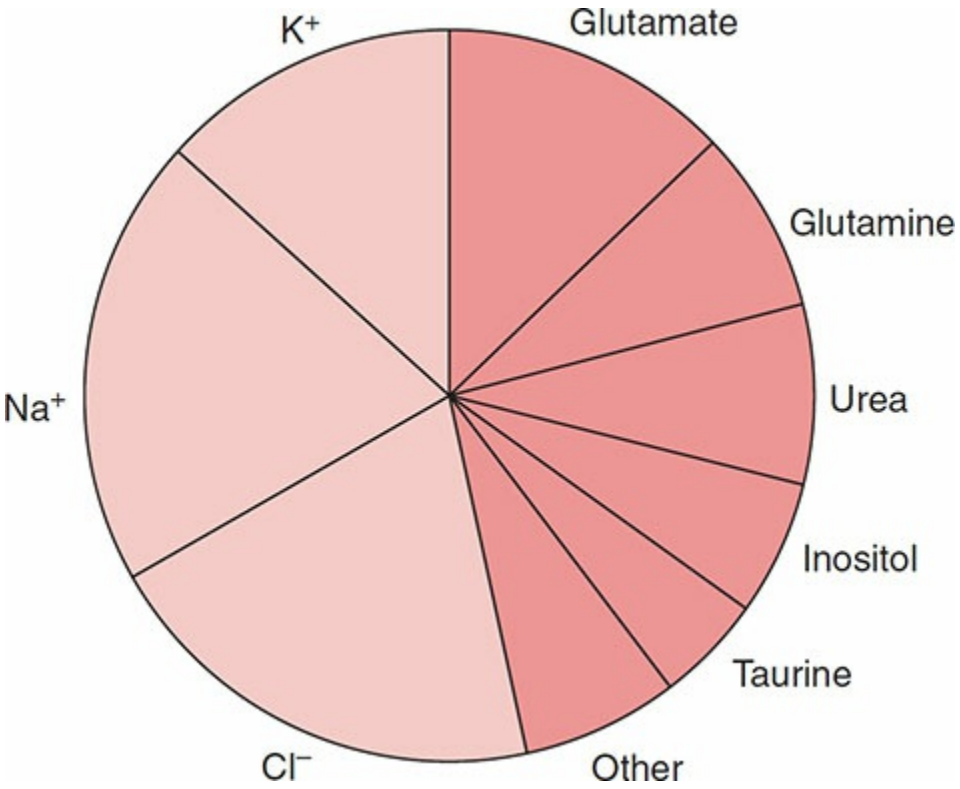

Figure 1–17 Relative decreases in individual osmolytes during adaptation to chronic hyponatremia. The category "other" represents glycerilphosphorylcholine, urea, and several other amino acids. (Reproduced with permission of Annual Review from Gullans SR, Verbalis JG. Control of brain volume during hyperosmolar and hypoosmolar conditions. *Annu Rev Med.* 1993;44:289–301, with permission. 1993, Copyright © by Annual Reviews, http://www.annualreviews.org.)

Symptomatic Hyponatremia

Acute

Neurologic symptoms of acute hyponatremia are most commonly seen in premenopausal females in the postoperative state (102), elderly persons on thiazide diuretics, patients with psychogenic polydipsia (125), and marathon runners (119). It is generally agreed that these patients should be treated promptly. In this setting, the risks of cerebral edema and seizures outweigh any risk of rapid correction. The mortality rate in these patients is considerable, and those who survive frequently have residual neurologic signs and symptoms. The female preponderance of the syndrome is not fully understood. The volume adaptive response whereby the brain decreases its volume in acute hyponatremia may be inhibited by female hormones. A contribution of hypoxia also may be important, because when hypoxia is combined with hyponatremia in experimental animals; the volume adaptive response is abrogated, resulting in brain edema and increased mortality (126). Because the neurologic complications associated with acute symptomatic hyponatremia are devastating, these patients require prompt treatment with 3% NaCl (65,127,128). A rapid increment of 4 to 6 mEq/L appears to be sufficient to reverse cerebral edema (129). Thus, initial treatment should entail an infusion of 1 to 2 mL/kg over 60 minutes.

Chronic

Patients in whom the adaptive process to hyponatremia has occurred (>48 hours) are at risk of developing a demyelinating syndrome when treated rapidly (122,130). If the hyponatremia has taken more than 48 hours to evolve or if the duration is not known, correction should be undertaken with caution. Controversy exists as to whether it is the rate of correction or the magnitude of correction of hyponatremia that predisposes to neurologic complications. In clinical practice, it is difficult to dissociate these two variables since a rapid correction rate is usually accompanied by a greater absolute magnitude of correction over a given period of time. There are principles that have been in large measure agreed upon to guide treatment (65,128,131):

1. The goal in the first 24 hours is an increase in plasma sodium of 6 to 8 mEq/L
2. The limit that should not be exceeded is 12 mEq in the first 24 hours and 18 in the first 46 hours.
3. If these limits are exceeded, undertake steps to re-lower the plasma

sodium to comply with them.

Numerous formulas have been proposed to assess the effects of infusions (124) but the one proposed by Adrogue and Madias is the most widely employed (71). Although this formula is helpful in the initial treatment phase, it does not take into account ongoing renal and extrarenal losses (132), thus commonly resulting in correction larger than those predicted by the formula (133). The risk of overcorrection can be mitigated by the coadministration of DDAVP, thus preventing the excretion of a hypotonic urine (134). If the aforementioned limits are exceeded, re-lowering of plasma sodium can be achieved by the infusion of dextrose and water and the administration of DDAVP (134).

The rapid increase in serum osmolality leads to a greater degree of cerebral water loss than a previously normonatremic brain (122) and makes the development of a demyelinating process more likely. More recent studies have pointed to astrocytes as the cells that are the target for osmotic injury by the generation of cytokines in the process leading to demyelination (135,136). Although small demyelinating lesions produce minimal symptoms, patients with more extensive diseases have flaccid quadriplegia, dysphagia, and dysarthria. A more recent multicenter survey has shown that the disorder is not uniformly fatal and sometimes leaves only mild residua (137). The risk factors that predispose to osmotic demyelination have not been fully delineated. Patients with underlying liver disease, alcoholism receiving thiazide diuretics, malnutrition, severe hypokalemia, and serum Na <105 mEq/L are more prone to this complication.

Asymptomatic

Patients with asymptomatic hyponatremia invariably have a chronic component to their disease (138). Despite the fact that these patients are asymptomatic and the hyponatremia frequently mild, several studies have shown that the hyponatremia is associated with increased mortality (139–142). Although many may appear to be asymptomatic gait disturbances comparable to those seen in subjects with toxic levels of alcohol, that reverse upon correction of the hyponatremia (143) have been reported. This gait disturbance coupled with subtle neurocognitive deficits (143,144) results in an increased risk of falls and fractures (145).

Water restriction has been the cornerstone of the treatment of

“asymptomatic” hyponatremic patients. The degree of the restriction depends on the severity of the diluting impairment, which can be assessed by urinary electrolyte measurements (146). When the diluting defect is severe, as reflected by a ratio of urinary Na plus urinary potassium to plasma sodium that is greater than 1, almost no amount of fluid restriction will be effective. Likewise, a urinary osmolality greater than 500 mOsm/kg also predicts poor response to water restriction (116). In a recently hyponatremia registry observation, the increment in plasma sodium observed in patients placed on water restriction did not have a significant response to the intervention (147). Furthermore, water restriction is difficult to enforce over a long time. Therefore, approaches that instead enhance water excretion, such as agents that antagonize the renal action of AVP have been employed. Demeclocycline is more effective and safer than lithium in the treatment of SIADH, but is frequently accompanied by gastrointestinal intolerance. Moreover, in hyponatremic subjects with cirrhosis demeclocycline causes nephrotoxicity and thus should be avoided.

Urine flow and thereby water excretion can be increased by increasing solute excretion (148). A dose of 30 to 60 g of urea per day can successfully treat the syndrome. A similar benefit occurs with the use of furosemide (40 mg/day) with a high salt intake (200 mEq/day).

The development of orally active, nonpeptide antagonists of the hydroosmotic effect of AVP (Vaptans) provides a pathophysiologically fitting therapeutic tool in the management of patients with hyponatremia (149,150). Table 1-12 summarizes the pharmacokinetics and pharmacodynamics of the two antagonists presently approved in the United States, Canada, and Europe. Several studies have demonstrated that they can induce a water diuresis and increase serum sodium in patients with SIADH (151–154) as well as with cirrhosis (153,155–157) and congestive heart failure (79,153,158). Their effectiveness is confirmed by two meta-analyses (159,160). The oral agent (tolvaptan) maintains its effect over prolonged administration (161,162). In the only head to head study with another agent, its effectiveness was comparable to that of urea (162). Urea, however, is not readily available as a USP preparation and is not well tolerated for its gastrointestinal symptoms. Tolvaptan is well tolerated, but can cause increased thirst, overcorrection of plasma sodium, and at higher doses potential hepatic toxicity (163). Table 1-12 summarizes the pharmacokinetics and pharmacodynamics of the two antagonists presently approved in the United States, Canada, and Europe. Although they differ in their relative V_1/V_2 selectivity, they all lower

urinary osmolality, and have no effect on sodium and potassium excretion. Conivaptan, a V_1/V_2 antagonist, is FDA approved for intravenous use (164) for the treatment of hospitalized patients with euvolemic or hypervolemic hyponatremia. Its use is limited to 4 days because it is a potent CYP3A4 inhibitor. In view of its V_1 antagonistic properties, its use in patients with liver disease is probably not advisable. Tolvaptan is a weaker CYP inhibitor and has approval for oral use after initiation in a hospital setting to ensure that overcorrection does not occur. The potential for overcorrection needs to be considered and there are scattered case reports, but in all of them, the concomitant use of 3% NaCl was a contributory factor (165). This is a combination that should be shunned. Although still lacking in hard outcomes such as morbidity, mortality, hospitalization length, and despite being of high cost, V_2 receptor antagonists are an attractive alternative to water restriction and are likely to emerge as the agents of choice in the treatment of hyponatremia (166).

Table 1–12 Inhibitory Constants and Pharmacokinetics of Two Vasopressin Antagonists

Variable	Conivaptan	Tolvaptan
Inhibitory Constant of Vasopressin Antagonist[a]		
V_1 receptor (nM)	6.3	12.3
V_2 receptor (nM)	1.1	0.4
V_2:V_1 selectivity ratio	5.7	29.0
Pharmacokinetics of Vasopressin Antagonists[b]		
Dose	Intravenous administration: 40 mg daily for 4 d	Oral administration: 15–60 mg daily
Half-life (h)	6–10	6–8
Time to maximum aquaresis after administration (h)	2	2
Protein binding (%)	95–99	99
Oral bioavailability (%)	40–50	40–50
Primary metabolism	CYP3A4	CYP3A4
Urinary excretion (%)	<1	<5

[a]Data are adapted from Tahara et al. (167) and Yamamura et al. (168). The

inhibitory constant (Ki) is the inhibitor level that produces half the maximal rate, so a smaller Ki value indicates a more potent inhibitor.

[b]Data are adapted from Costello-Boerrigter et al. (169).

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CHAPTER 2

Renal Sodium Excretion, Edematous Disorders, and Diuretic Use

Robert W. Schrier

An understanding of body fluid volume regulation, as modulated by renal sodium and water excretion, is critical for the practice of clinical medicine. Knowledge of the intrarenal and extrarenal factors affecting renal sodium excretion is important to comprehend the mechanism of body fluid volume regulation in health and disease because the sodium ion is the primary determinant of extracellular fluid (ECF) volume. In this regard, the edematous disorders—cardiac failure, liver disease, and the nephrotic syndrome—present a particular challenge to our understanding of body fluid volume regulation. In normal humans, if the ECF volume is expanded by the administration of isotonic saline, then the kidney excretes the excess amount of sodium and water in the urine, thus returning the ECF volume to normal. However, in these edematous states, avid renal sodium and water retention persists despite expansion of ECF volume and the presence of total body sodium and water excess. In circumstances where advanced kidney disease is present and renal function and excretory capacity are diminished (e.g., acute or chronic intrinsic renal failure), it is obvious why the decreased glomerular filtration rate (GFR) may be associated with retention of sodium and water to the point of pulmonary and/or peripheral edema. However, it is clear that the integrity

of the kidney as the ultimate effector organ of body fluid volume regulation is intact in patients with heart failure or liver disease and some patients with the nephrotic syndrome. Thus, the kidney must be responding to extrarenal signals from the afferent limb of a volume regulatory system in these edematous disorders. The study of these edematous disorders has led to our proposal of a unifying hypothesis of body fluid volume regulation that applies to both health and disease (1–8). The purpose of this chapter is to review the afferent and efferent mechanisms that determine renal sodium and water handling, particularly in the context of the edematous disorders, and discuss the treatment of edema with diuretic agents.

Sodium Ion as Determinant of ECF Volume

Sodium ions reside primarily in the ECF compartment to which they are extruded from cells by active transport mechanisms. These transport processes result in an intracellular sodium concentration of 10 mEq/L and an ECF sodium concentration of 145 mEq/L. The sodium ion and its major anions, chloride and bicarbonate, constitute >90% of the total solute in the ECF space. Thus, total body sodium and its accompanying anions are the osmotically active solutes that are the major determinants of ECF volume. In turn, the regulation of sodium balance is determined by the relationship among sodium intake, extrarenal sodium loss, and renal sodium excretion. Practically, renal sodium excretion may be considered to be the primary determinant of sodium balance because the kidney is able to excrete virtually sodium-free urine as well as rapidly excrete large sodium loads in response to diminished or increased sodium intakes, respectively.

A positive sodium balance is associated with increased amounts of sodium, located predominantly in the ECF compartment. Because cellular membranes are freely permeable to water, the osmotic gradient created by the addition of ECF sodium causes water to move from cells into the ECF compartment, thus expanding ECF volume. In addition, an increase in ECF osmolality stimulates the hypothalamic thirst center and leads to increased fluid intake and also releases arginine vasopressin (AVP) from the posterior pituitary, which decreases renal water excretion by increasing the water permeability of collecting duct epithelium (9). The latter two effects of an increased ECF osmolality result in a positive water balance, and the combined influence of positive sodium and water balances leads to further expansion of ECF volume. If this expansion of ECF is of sufficient

magnitude, then an alteration of the Starling forces that govern the transfer of fluid from the vascular compartment to the surrounding interstitial spaces occurs and edema results (10). Conversely, a negative sodium balance results in a depletion of ECF volume. A decrease in ECF volume may result in a parallel decline in plasma volume. Maintenance of ECF volume and plasma volume is necessary for adequate circulation and survival of the organism. Thus, renal sodium and water retention is clearly appropriate in situations of ECF volume depletion. However, in edematous disorders, continued renal sodium and water retention despite total body sodium and water excess defines a paradoxical clinical situation.

It is worth mentioning that the osmolality of ECF is regulated by the AVP–thirst–renal axis (as discussed in depth in Chapter 1). However, the osmolality of the ECF is not a reliable index of ECF volume. ECF volume and its determinant total body sodium are best assessed by physical examination and determination of urinary sodium concentration. For example, a finding of generalized edema suggests an expanded ECF volume and increased total body sodium. Conversely, orthostatic tachycardia and/or hypotension, flat neck veins, and decreased skin turgor suggest depletion of ECF volume and decreased total body sodium. In fact, alterations in the osmolality of the ECF can occur in association with normal, increased, or decreased ECF volume (Chapter 1).

In summary, the control of ECF volume is dependent on the regulation of sodium balance. The kidneys play the pivotal role in the regulation of sodium balance and therefore of ECF volume homeostasis. In certain edema-forming states associated with a normal GFR, the kidney retains sodium and water despite expansion of the ECF volume and total body sodium and water. A knowledge of the afferent (“sensor”) and efferent (“effector”) mechanisms of sodium and water retention associated with the edematous disorders forms the basis of our understanding of body fluid volume regulation.

Afferent Mechanisms Involved in Body Fluid Volume Regulation

THE CONCEPT OF “EFFECTIVE BLOOD VOLUME” OR WHAT COMPARTMENT IS SENSED?

If the afferent receptors of body fluid volume regulation primarily sense total blood volume, then the kidneys of edematous patients should increase

their excretion of sodium and water since their total blood volumes are increased. However, as mentioned, this does not occur in patients with advanced cardiac failure, liver disease, or the nephrotic syndrome. Thus, there must be some body fluid compartment that is still "underfilled"—even in the presence of expansion of total ECF and blood volumes—and comprises the afferent limb of renal sodium and water retention in patients with edematous disorders. In 1948, Peters coined the enigmatic term *effective blood volume* as a reference to such an underfilled body fluid compartment (11). Accordingly, extrarenal signals must be initiated by this decrease in effective blood volume, which enhances tubular sodium and water reabsorption by the otherwise normal kidney. In this regard, it is clear that renal sodium and water retention can occur in patients with cardiac failure or cirrhosis and in some patients with the nephrotic syndrome before any diminution in GFR.

Borst and deVries (12) first suggested cardiac output as the primary modulator of renal sodium and water excretion. In this context, the level of cardiac output would constitute the effective blood volume and thus serve as the primary stimulus for renal sodium and water retention in patients with edematous disorders. Although this concept is appealing, substantial renal sodium and water retention may occur in the presence of an increase in cardiac output. For example, a significant elevation in cardiac output may occur in the presence of avid renal sodium and water retention and expansion of ECF volume in association with cirrhosis, pregnancy, arteriovenous (AV) fistulas, and other causes of high-output cardiac failure, such as thyrotoxicosis and beriberi. Consequently, there must exist some other or additional determinant(s) of effective blood volume.

PRIMACY OF THE ARTERIAL CIRCULATION IN VOLUME REGULATION

The unifying hypothesis of body fluid volume regulation in health and disease states that the fullness of the arterial vascular compartment or the so-called effective *arterial* blood volume (EABV) is the primary determinant of renal sodium and water excretion (1–8). In a 70-kg man, total body water approximates 42 L, of which only 0.7 L (1.7% of total body water) resides in the arterial circulation. From a teleologic viewpoint, it is attractive to propose that the primacy for regulation of renal sodium and water excretion, and body fluid volume homeostasis, is modulated by the smallest body fluid compartment—thus endowing the system with exquisite sensitivity to relatively small changes in body fluid volume.

Another advantage of the integrity of the arterial circulation constituting the main afferent sensing compartment for body fluid volume regulation is that perfusion of the vital organs is dependent on the arterial circulation. As a result, total ECF, interstitial fluid, or total intravascular volumes are not primary determinants of renal sodium and water excretion, and the venous component of intravascular volume likewise is excluded as the primary determinant of sodium and water excretion, because all of these body fluid compartments may be expanded while the renal sodium and water retention persists in edematous patients. It is acknowledged, however, that there are experimental and clinical circumstances in which selective rises in right and left atrial pressure stimulate the release of atrial natriuretic peptide (ANP) (13) or suppression of AVP (14), respectively, which may enhance sodium and water excretion. These events, however, must be subservient to the more potent determinants of the arterial circulation because the patient with advanced left or right ventricular dysfunction, or both, exhibits avid sodium and water retention despite markedly elevated atrial and ventricular pressures.

CARDIAC OUTPUT AND SYSTEMIC ARTERIAL RESISTANCE AS THE DETERMINANTS OF THE FULLNESS OF THE ARTERIAL CIRCULATION AND RENAL SODIUM AND WATER EXCRETION

The EABV is a measure of the adequacy of arterial blood volume to “fill” the capacity of the arterial circulation. Normal arterial filling exists when the ratio of cardiac output to systemic vascular resistance maintains venous return and cardiac output at normal levels. Thus, arterial underfilling may be initiated by either a decrease in cardiac output or a fall in systemic arterial resistance (i.e., arterial vasodilatation, which increases the holding capacity of the arterial vascular tree). Arterial underfilling results in unloading of high-pressure baroreceptors with subsequent activation of the three major neurohormonal vasoconstrictor systems—namely, the sympathetic nervous system, the renin–angiotensin–aldosterone system, and the nonosmotic release of AVP—which diminish renal hemodynamics and promote renal sodium and water retention. This hypothesis accounts for the initiation of sodium and water retention in low- and high-output cardiac failure, liver disease, and other states of arterial underfilling (Figs. 2-1 and 2-2).

AFFERENT VOLUME RECEPTORS

As mentioned, the afferent volume receptors for such a volume regulatory system must reside in the arterial vascular tree, such as the high-pressure baroreceptors in the carotid sinus, aortic arch, left ventricle, and juxtaglomerular apparatus. Although the low-pressure volume receptors of the thorax (cardiac atria, right ventricle, and pulmonary vessels) must be of some importance to the volume regulatory system (15,16), there is considerable evidence that arterial receptors can predominate over low-pressure receptors in volume control in mammals.

High-Pressure Volume Receptors

In humans, the presence of volume-sensitive receptors in the arterial circulation was first suggested by Epstein et al. (17) based on observations made in patients with traumatic AV fistulas. Closure of traumatic AV fistulas was associated with an immediate increase in renal sodium excretion independent of concomitant changes in either GFR or renal blood flow (RBF) (17). Closure of AV fistulas is associated with a decreased rate of emptying of the arterial blood into the venous circulation, as demonstrated by closure-induced increases in diastolic arterial pressure and decreases in cardiac output (17). Further evidence implicating the relative “fullness” of the arterial vascular tree as being the major sensor in modulating renal sodium excretion can be found in denervation experiments. In these studies, surgical or pharmacologic interruption of sympathetic efferent neural pathways emanating from high-pressure areas inhibited the natriuretic response to volume expansion (18–20). Moreover, reduction of pressure or stretch at the carotid sinus, similar to that produced by decreased cardiac output or arterial hypotension, has been shown to activate the sympathetic nervous system and promote renal sodium and water retention (21). High-pressure baroreceptors also appear to be important factors in regulating nonosmotic release of AVP and thus renal water excretion (22). One of the best-defined high-pressure receptors that are known to act in an appropriate manner to maintain constancy of the EABV is the renal afferent arteriolar baroreceptor (i.e., juxtaglomerular apparatus). This baroreceptor is an important factor in the control of renal renin secretion and consequently angiotensin II formation and aldosterone synthesis and release (23). The vasoconstrictor and sodium-retaining effects of angiotensin II and sodium-retaining effect of aldosterone then act to restore the fullness of the arterial circulation.

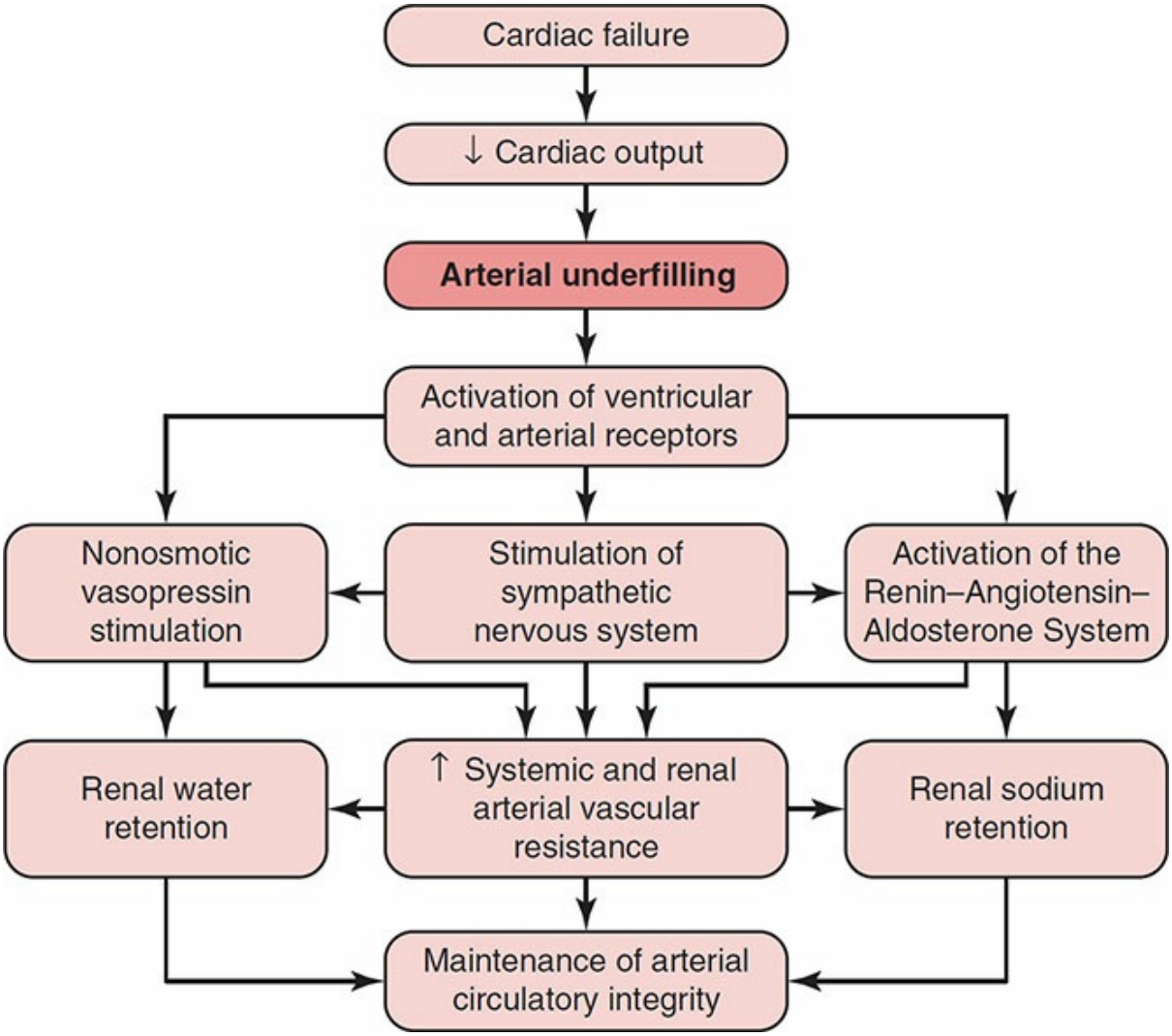

Figure 2–1 Clinical conditions in which a decrease in cardiac output causes arterial underfilling with resultant neurohumoral activation and renal sodium and water retention. (From Schrier RW. Decreased effective blood volume in edematous disorders: what does this mean? *J Am Soc Nephrol*. 2007;18(7): 2028–2031, permission conveyed through Copyright Clearance Center, Inc.)

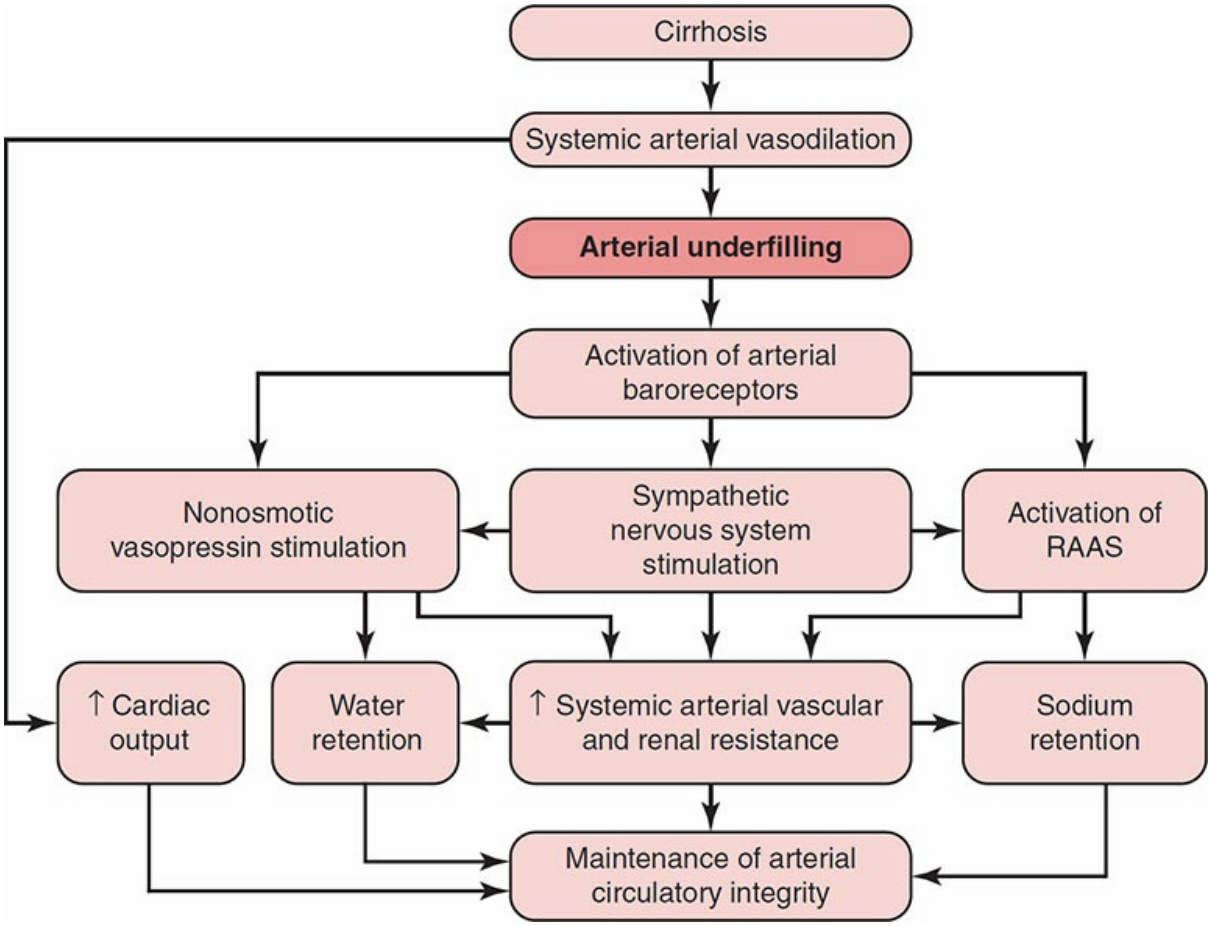

Figure 2–2 Clinical conditions in which systemic arterial vasodilation causes arterial underfilling with resultant neurohumoral activation and renal sodium and water retention. (From Schrier RW. Decreased effective blood volume in edematous disorders: what does this mean? *J Am Soc Nephrol*. 2007;18(7):2028–2031, permission conveyed through Copyright Clearance Center, Inc.)

Low-Pressure Volume Receptors

Low-pressure sensors also may have an important role to play in body fluid volume regulation because the more compliant venous side of the circulation contains up to 85% of the total blood volume at any given time (Table 2-1). In fact, a variety of maneuvers that decrease thoracic venous return, such as prolonged standing (24), lower-extremity tourniquets (25,26), and positive pressure breathing (27), are associated with diminished renal sodium excretion. Conversely, maneuvers that augment venous filling, such as recumbency (28) and negative pressure breathing (29), are associated with increased renal sodium excretion. Moreover, a direct correlation between renal sodium excretion and left atrial pressure has been demonstrated in dogs, suggesting a role for an atrial receptor as one type of intrathoracic sensor (30). Immersion in water to the neck or so-

called head-out water immersion results in a pressure gradient from 80 mm Hg at the foot to 0 mm Hg at water level. This maneuver increases venous return to the heart. In response to head-out water immersion, a profound increase in renal excretion of salt and water occurs independent of major changes in either GFR or renal hemodynamics (31). As first suggested by Gauer et al. (29) and Henry et al. (32), physiologically significant left atrial receptors have been shown to contribute to ECF volume regulation by exerting nonosmotic control over AVP secretion and thus over renal water excretion. In addition, the atria have been demonstrated to be the site for synthesis, storage, and release of vasoactive and natriuretic humoral agents (33,34).

Thus, increased filling of the thoracic vascular and cardiac atria would be expected to signal the kidney to increase urinary sodium excretion in order to return the blood volume to normal. However, in the setting of chronic heart failure, renal sodium and water retention occur despite increased atrial pressure, which loads the low-pressure baroreceptors. Thus, in low-output chronic heart failure, diminished cardiac output must exert the predominant effect via unloading of high-pressure arterial baroreceptors. Chronic studies in animals employing experimental tricuspid insufficiency (35) further support this hypothesis. The increase in right atrial pressure was associated with avid renal sodium retention in these animal models. However, a concomitant fall in cardiac output likely explains the sodium retention.

Zucker et al. (36) have demonstrated that the inhibition of renal sympathetic nerve activity that is seen during acute left atrial distention is lost during chronic heart failure in dogs. Moreover, a decrease in cardiac preload fails to produce the expected parasympathetic withdrawal and sympathetic activation in humans with heart failure (37). These findings are also consistent with the observation of a strong positive correlation between left atrial pressure and coronary sinus norepinephrine, a marker of cardiac adrenergic activity, in patients with chronic heart failure (38). Taken together, these findings suggest that the normal inhibitory control of sympathetic activation accompanying increased atrial pressures is lost in heart failure patients and somehow may even be converted to a stimulatory signal.

Table 2–1 Body Fluid Distribution

Compartment	Amount	Volume in 70-kg Man (L)

Total body fluid	60% of body weight	42
Intracellular fluid	40% of body weight	28
Extracellular fluid	20% of body weight	14
Interstitial fluid	Two-thirds of extracellular fluid	9.4
Plasma fluid	One-third of extracellular fluid	4.6
Venous fluid	85% of plasma fluid	3.9
Arterial fluid	15% of plasma fluid	0.7

In summary, the afferent or sensor mechanisms for sodium and water excretion may be preferentially located on the arterial side of the circulation where diminished fullness of the arterial vascular tree owing to decreased cardiac output or systemic arterial vasodilation results in unloading of high-pressure receptors and subsequent renal sodium and water retention. Reflexes from low-pressure volume receptors may also be altered so as to influence renal sodium and water handling. In any event, changes in systemic and renal hemodynamics and activation of various neurohormonal systems largely comprise the efferent limb of the volume regulatory system.

Efferent Mechanisms Involved in Body Fluid Volume Regulation

THE NEUROHORMONAL RESPONSE TO ARTERIAL UNDERFILLING

Arterial underfilling secondary to a diminished cardiac output or systemic arterial vasodilation elicits a number of compensatory neurohormonal responses that act to maintain the integrity of the arterial circulation by promoting systemic vasoconstriction as well as expansion of the ECF volume through renal sodium and water retention. As noted, the three major neurohormonal vasoconstrictor systems activated in response to

arterial underfilling are the sympathetic nervous system, renin–angiotensin–aldosterone system, and nonosmotic release of AVP. Baroreceptor activation of the sympathetic nervous system appears to be the primary integrator of the hormonal vasoconstrictor systems involved in the volume control system because the nonosmotic release of AVP involves sympathetic stimulation of the supraoptic and paraventricular nuclei in the hypothalamus (39), and activation of the renin–angiotensin–aldosterone system involves renal β-adrenergic stimulation (40). Thus, in low-output cardiac failure, diminished integrity of the arterial circulation as determined by decreased cardiac output causes unloading of arterial baroreceptors in the carotid sinus and aortic arch. Systemic arterial vasodilation produces unloading of these arterial baroreceptors in the setting of high-output cardiac failure, cirrhosis, and other states of arterial underfilling. This baroreceptor inactivation results in diminution of the tonic inhibitory effect of afferent vagal and glossopharyngeal pathways to the central nervous system (CNS) and initiates an increase in sympathetic efferent adrenergic tone with subsequent activation of the renin–-angiotensin–aldosterone system. Various counterregulatory, vasodilatory hormones may also be activated in heart failure, including natriuretic peptides and vasodilating renal prostaglandins. Activation of these various neurohormonal vasoconstrictor and vasodilator systems substantially determines renal sodium and water handling in the edematous disorders and comprises a major part of the efferent limb of body fluid volume regulation. The pathogenesis of sodium and water retention associated with cardiac failure, liver disease, and the nephrotic syndrome are now reviewed in the context of the unifying arterial underfilling hypothesis of body fluid volume regulation.

PATHOGENESIS OF SODIUM AND WATER RETENTION IN CARDIAC FAILURE

Sodium and water retention and resultant edema formation are cardinal features of chronic cardiac failure. In fact, the inability to excrete a sodium load has been used as an index of the presence of heart failure (41), and a defect in water excretion is encountered routinely in such patients (42). Two theories have been proposed to explain the renal response to cardiac failure. The “backward” theory of heart failure, proposed in 1832, suggests that increased venous hydrostatic pressure owing to increased ventricular filling pressures causes edema by promoting transudation of fluid from the intravascular to the interstitial compartment, resulting in edema formation

(43). The reduced intravascular volume then signals the kidneys to retain sodium and water, further exacerbating the venous hypertension and formation of edema. The alternative “forward” theory of cardiac failure suggests that a primary decrease in cardiac output activates afferent and efferent pathways and results in renal sodium retention (44). As pointed out by Smith (26), these theories are not mutually exclusive and both are operant in the pathophysiology of heart failure because both central venous hypertension and arterial underfilling are implicated in the afferent limb of body fluid volume regulation. Nevertheless, the dominant signal for sodium and water retention in cardiac failure appears to occur in the arterial circulation. Decreased cardiac output is the cause of the arterial underfilling in the case of low-output heart failure, whereas systemic arterial vasodilation initiates the afferent limb of sodium and water retention in high-output cardiac failure (Fig. 2-1).

Renal Hemodynamics in Cardiac Failure

Glomerular Filtration Rate

Many early investigators believed that the cause of sodium retention in heart failure was a decrease in GFR; however, studies failed to confirm such a correlation. In fact, GFR is often normal in early heart failure and may even be elevated in states of high-output cardiac failure. It is acknowledged, however, that the contribution of GFR to sodium balance is difficult to evaluate because very minute changes in GFR could lead to substantial changes in sodium excretion if absolute sodium reabsorption remained unchanged. Nevertheless, although GFR may be diminished in patients with advanced heart failure, an increase in tubular sodium reabsorption undoubtedly is an important cause of sodium and water retention in cardiac failure.

Renal Blood Flow

Heart failure is commonly associated with an increase in renal vascular resistance and a decrease in RBF. In general, RBF decreases in proportion to the decrease in cardiac output. Some investigators have also shown a redistribution of RBF from outer cortical nephrons to juxtamedullary nephrons in experimental heart failure (45). It was proposed that deeper nephrons with longer loops of Henle reabsorb sodium more avidly; thus, the redistribution of blood flow to these nephrons with heart failure would

result in renal sodium retention. However, other investigators have not been able to demonstrate such a redistribution of blood flow in other models of cardiac failure (46). Thus, the role of redistribution of RBF in the sodium retention of cardiac failure remains uncertain.

Filtration Fraction

Filtration fraction is often increased in heart failure because RBF falls as cardiac output decreases and GFR is preserved. An increase in filtration fraction results in increased protein concentration and oncotic pressure in the efferent arterioles and peritubular capillaries that surround the proximal tubules. Such an increase in peritubular oncotic pressure has been proposed to increase sodium and water reabsorption in the proximal tubule. These changes in renal hemodynamics and filtration fraction, which favor proximal tubular sodium reabsorption, are primarily a consequence of constriction of the efferent arterioles within the kidney. These renal hemodynamic changes are mediated mainly by activation of neurohormonal vasoconstrictor systems because both activation of renal nerves and increased circulating norepinephrine and angiotensin II have been implicated in efferent arteriolar vasoconstriction (47,48). In addition, decreased activity of such substances as vasodilating renal prostaglandins also may play a role in renal vasoconstriction (49).

Of note, micropuncture studies in dogs with vena caval constriction and AV fistulas have demonstrated the importance of distal nephron sites of increased sodium reabsorption. Increased filtration fraction primarily affects proximal tubular sodium reabsorption. Thus, although clearance and micropuncture studies in animals with heart failure have demonstrated increased sodium reabsorption in the proximal tubule (50), distal sodium reabsorption also seems to be involved. Furthermore, changes in filtration fraction have been observed in heart failure long before changes in sodium balance occur, questioning the dominance of peritubular factors and proximal reabsorption in the sodium retention of cardiac failure.

The Sympathetic Nervous System in Cardiac Failure

The sympathetic nervous system is unquestionably activated in patients with heart failure. Various studies have demonstrated elevated peripheral venous plasma norepinephrine concentrations in heart failure patients. Using tritiated norepinephrine in patients with advanced heart failure, Davis et al. (51) and Hasking et al. (52) have shown that both increased

norepinephrine secretion and decreased norepinephrine clearance contribute to the high venous plasma norepinephrine concentrations seen in these patients, suggesting that increased sympathetic activity is at least partially responsible for the elevated circulating plasma norepinephrine. We have demonstrated that the initial rise in plasma norepinephrine in heart failure is solely caused by increased norepinephrine secretion, providing evidence of increased sympathetic nervous system activity early in the course of cardiac failure (53). Moreover, plasma norepinephrine is increased in patients with asymptomatic left ventricular dysfunction (i.e., before the onset of overt heart failure) (54). Finally, studies employing peroneal nerve microneurography to directly assess sympathetic nerve activity to muscle have confirmed the presence of increased sympathetic activity in heart failure patients (55). Significantly, the degree of activation of the sympathetic nervous system—as assessed by the peripheral venous plasma norepinephrine concentration—has been correlated with poor prognosis in heart failure (56).

Activation of Renal Nerves

Renal nerves also are activated in human heart failure (52). Enhanced renal sympathetic activity may contribute to the avid sodium and water retention in heart failure by promoting renal vasoconstriction, stimulation of the renin–angiotensin–aldosterone system, and direct effects on the proximal tubule epithelium. Indeed, intrarenal adrenergic blockade has been shown to cause a natriuresis in experimental heart failure (57). In addition, in rats, renal nerve stimulation has been demonstrated to produce approximately a 25% reduction in sodium excretion and urine volume (58). The diminished renal sodium excretion that accompanies renal nerve stimulation may be mediated by at least two mechanisms. As already discussed, studies performed in rats have demonstrated that norepinephrine-induced efferent arteriolar constriction alters peritubular hemodynamic forces in favor of increased tubular sodium reabsorption (47). In addition, renal nerves have been shown to exert a direct influence on sodium reabsorption in the proximal convoluted tubule (58).

Bello-Reuss et al. (58) have demonstrated this direct effect of renal nerve activation to enhance proximal tubular sodium reabsorption in whole-kidney and individual nephron studies in rats. In these animals, renal nerve stimulation produced an increase in the tubular fluid–to–plasma inulin concentration ratio in the late proximal tubule, an outcome of increased fractional sodium and water reabsorption in this segment of

the nephron. Hence, increased renal nerve activity may promote sodium retention by a mechanism independent of changes in renal hemodynamics. On the other hand, sodium retention persists in dogs with denervated transplanted kidneys and chronic vena caval constriction. Moreover, renal denervation does not prevent ascites in dogs with chronic vena caval constriction (59). Thus, renal nerves probably contribute but do not fully account for the avid sodium retention of heart failure.

The Renin–Angiotensin–Aldosterone System in Cardiac Failure

The renin–angiotensin–aldosterone system also is activated in heart failure, as assessed by plasma renin activity (PRA) (60). Renin acts on angiotensinogen to produce angiotensin I, which is then converted by angiotensin-converting enzyme (ACE) to angiotensin II. In heart failure, the resultant increased plasma concentration of angiotensin II exerts important circulatory effects, including peripheral arterial and venous vascular constriction, renal vasoconstriction, and cardiac inotropism. Activation of angiotensin receptors on the proximal tubule epithelium directly stimulates the Na^+/H^+ exchanger 3 and thereby increases sodium reabsorption (61). Angiotensin II also acts to promote the secretion of the sodium-retaining hormone aldosterone by the adrenal cortex and in positive-feedback stimulation of the sympathetic nervous system. Activation of this hormonal system may promote sodium retention in the kidney via several mechanisms, as discussed next. Moreover, like adrenergic activation, stimulation of the renin–angiotensin–aldosterone system is associated with an unfavorable prognosis in heart failure (62).

Renal Effects of Increased Angiotensin II and Aldosterone

Angiotensin II may contribute to the sodium and water retention in heart failure through direct and indirect effects on proximal tubular sodium reabsorption and, as mentioned, by stimulating the release of aldosterone from the adrenal gland. Angiotensin II causes preferential renal efferent arteriolar constriction, resulting in decreased RBF and an increased filtration fraction. As with renal nerve stimulation, this results in increased peritubular capillary oncotic pressure and reduced peritubular capillary hydrostatic pressure, which favors the reabsorption of sodium and water in the proximal tubule (48). Moreover, as noted, angiotensin II has been shown to enhance sodium reabsorption in the proximal tubule (63). In a study of the rat proximal tubule, Liu and Cogan (63) demonstrated

increased tubular sodium chloride reabsorption during the infusion of angiotensin II, whereas the angiotensin II receptor antagonist, saralasin, decreased proximal tubular sodium chloride reabsorption. Finally, in a report from Abassi et al. (64), the administration of the angiotensin II receptor antagonist, losartan, to decompensated sodium-retaining rats with heart failure secondary to AV fistulas produced a marked natriuresis. Although proximal tubular sodium handling was not examined in this investigation, the observation that losartan restored renal responsiveness to ANP is consistent with a losartan-induced increase in the delivery of sodium to the distal tubular site of ANP action. The role of distal tubular sodium delivery in the renal sodium retention of heart failure is discussed later.

Watkins et al. (65) studied a conscious dog model of heart failure in order to define more precisely the role of the renin–angiotensin–aldosterone axis in cardiac failure. Using either partial constriction of the pulmonary artery or thoracic inferior vena cava (TIVC), these workers acutely produced a low cardiac output state characterized by reduced blood pressure, increased PRA and aldosterone concentrations, and renal sodium retention. As plasma volume and body weight increased over several days, the aforementioned variables all returned toward control levels. During the initial hyperreninemic period, a single injection of an ACE inhibitor significantly lowered blood pressure. Also, chronic administration of the converting enzyme inhibitor prevented a rise in aldosterone and prevented 30% of the sodium retention and subsequent volume expansion. These studies lend support to the hypothesis that aldosterone is an important factor in the pathogenesis of cardiac edema and suggest that angiotensin II plays an important physiologic role in heart failure by supporting blood pressure because of its vasoconstrictor effect and maintaining blood volume secondary to the sodium-retaining effects of angiotensin II and aldosterone. It likewise becomes clear that, depending on the status of cardiac decompensation and plasma volume, the patient with heart failure may have a high or normal PRA and aldosterone level. This may explain some of the controversy that existed regarding the levels of these hormones in patients with heart failure.

A further role for renin–angiotensin–aldosterone system activation in the sodium retention of human heart failure is supported by the finding that urinary sodium excretion inversely correlates with PRA and urinary aldosterone excretion in heart failure patients (66). However, the administration of an ACE inhibitor (ACEI) during heart failure does not consistently increase urinary sodium excretion in spite of a consistent fall

in plasma aldosterone concentration (67). The simultaneous fall in blood pressure caused by decreased circulating concentrations of angiotensin II, however, may activate hemodynamic and neurohormonal mechanisms that could obscure the natriuretic response to lowered angiotensin II and aldosterone concentrations. Support for this hypothesis comes from the study performed by Hensen et al. (68). We examined the effect of the specific aldosterone antagonist, spironolactone, on urinary sodium excretion in patients with heart failure who were withdrawn from all medications before study. Avid sodium retention occurred in all patients throughout the period before aldosterone antagonism. During therapy with spironolactone (200 mg b.i.d.), all heart failure patients exhibited a significant increase in urinary sodium excretion and reversal of the positive sodium balance (Fig. 2-3). Moreover, the urinary sodium-to-potassium concentration ratio significantly increased during spironolactone administration, consistent with a decrease in aldosterone action in the distal nephron. Of note, PRA and norepinephrine increased and ANP decreased during the administration of spironolactone. Thus, this investigation demonstrates reversal of the sodium retention of heart failure with the administration of an aldosterone antagonist, despite further activation of various antinatriuretic influences, including stimulation of the renin–angiotensin and sympathetic nervous systems, and supports a role for aldosterone in the renal sodium retention. A prospective trial, Randomized Aldactone Evaluation Study (RALES), has shown improved survival of heart failure patients receiving 25 mg/day of spironolactone (a competitive inhibitor of aldosterone) (69). This effect of spironolactone in the RALES investigation was found to be independent of any change in sodium balance. An effect of spironolactone to block the effect of aldosterone-mediated cardiac fibrosis has been suggested as the mediator of this improved survival response. Natriuretic doses of spironolactone rarely have been used in patients with heart failure. One study was performed in congestive heart failure (CHF) patients receiving low-dose ACEIs who had diuretic resistance. These patients demonstrated a natriuresis with a daily dose of 100 mg of spironolactone (70). The Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (EPHESUS) demonstrated reduced mortality after acute myocardial infarction (71).

In the Acute Decompensated Heart Failure Registry (ADHERE), decompensated CHF patients resistant to oral diuretics were hospitalized and 90% were given intravenous diuretics. Forty-two percent of these patients were discharged with unresolved symptoms, 50% lost ≤5 lb, and

20% actually gained weight. Approximately 25% to 30% of CHF patients become resistant to diuretics and, as discussed later, secondary hyperaldosteronism is an important factor in such diuretic resistance (72).

Nevertheless, natriuretic doses of mineralocorticoid antagonists may not be part of the therapeutic armamentarium for heart failure, primarily because of the fear of hyperkalemia (73). Many of these CHF patients are receiving ACEIs or angiotensin receptor blockers (ARBs) and/or β-blockers, which predispose to hyperkalemia. Whether low-potassium diet, sodium polystyrene sulfonate (Kayexalate), and potassium-losing diuretics may avoid the occurrence of hyperkalemia during use of natriuretic doses of mineralocorticoid antagonists has not been studied. Given the challenge of treating cardiac patients with acute decompensation, as noted in the ADHERE registry, inhibiting the secondary hyperaldosteronism in sodium-retaining CHF patients who are diuretic resistant needs to be undertaken. Isotonic removal of sodium in CHF patients with ultrafiltration is another therapeutic approach. Fluid removal in CHF patients with ultrafiltration or diuretics can improve cardiac and renal function in addition to treating pulmonary congestion and edema. The mechanisms are shown in Figure 2-4 (74).

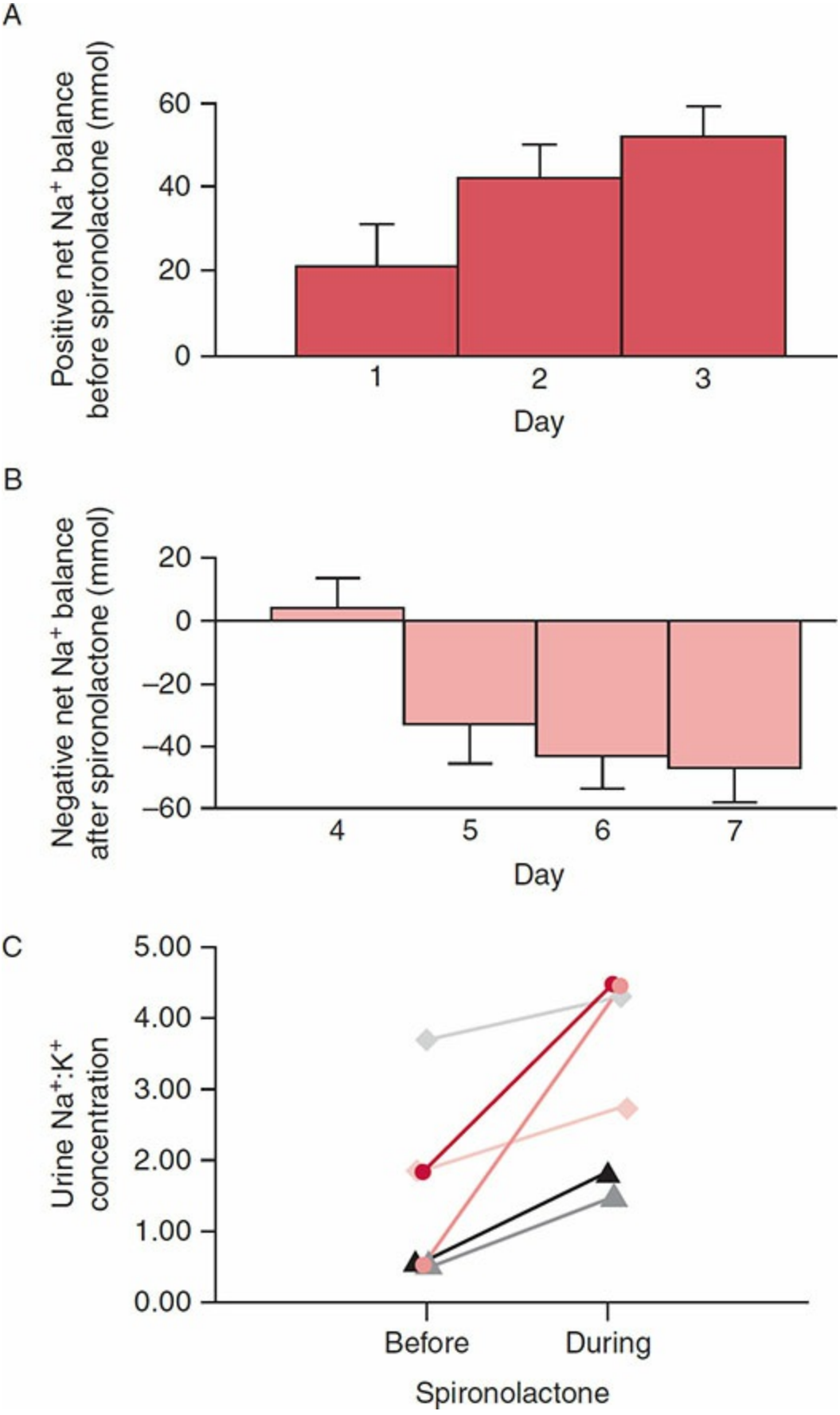

Figure 2–3 Diuretics, digoxin, and ACEI were withdrawn 4 days before admission to

General Clinical Research Center. The subjects were placed on a constant daily diet of 100 mEq sodium and 60 mEq potassium. (**A**) Upper panel demonstrates the positive cumulative sodium balance in the six patients (four ischemic heart disease, one idiopathic cardiomyopathy, and one aortic valvular disease). (**B**) Middle panel demonstrates in the same patients the significant negative cumulative sodium balance during 200 mg b.i.d. spironolactone ($P < 0.01$). (**C**) Lower panel demonstrates the increase in urine Na^+:K^+ concentration ratio during spironolactone in all six patients ($P < 0.05$), a finding compatible with aldosterone antagonism. Mean plasma potassium increased from 3.86 ± 0.2 to 4.1 ± 0.2 mEq/L during spironolactone treatment ($P < 0.05$). Mean systolic blood pressure (112 ± 7 mm Hg vs. 110 ± mm Hg, NS) and creatinine clearance (87 ± 7 mL/min vs. 87.2 ± 8 mL/min, NS) did not change with spironolactone treatment. Plasma hANP decreased significantly with spironolactone (147 ± 58 mg/L vs. 83 ± 30 mg/L, $P < 0.05$). Fluid intake was not restricted and a mean of 2 kg weight loss occurred. (From Bansal S, Lindenfeld J, Schrier RW. Sodium retention in heart failure and cirrhosis: potential role of natriuretic doses of mineralocorticoid antagonist? *Circ Heart Fail*. 2009;2(4):370–376, with permission from Wolters Kluwer Health, Inc.)

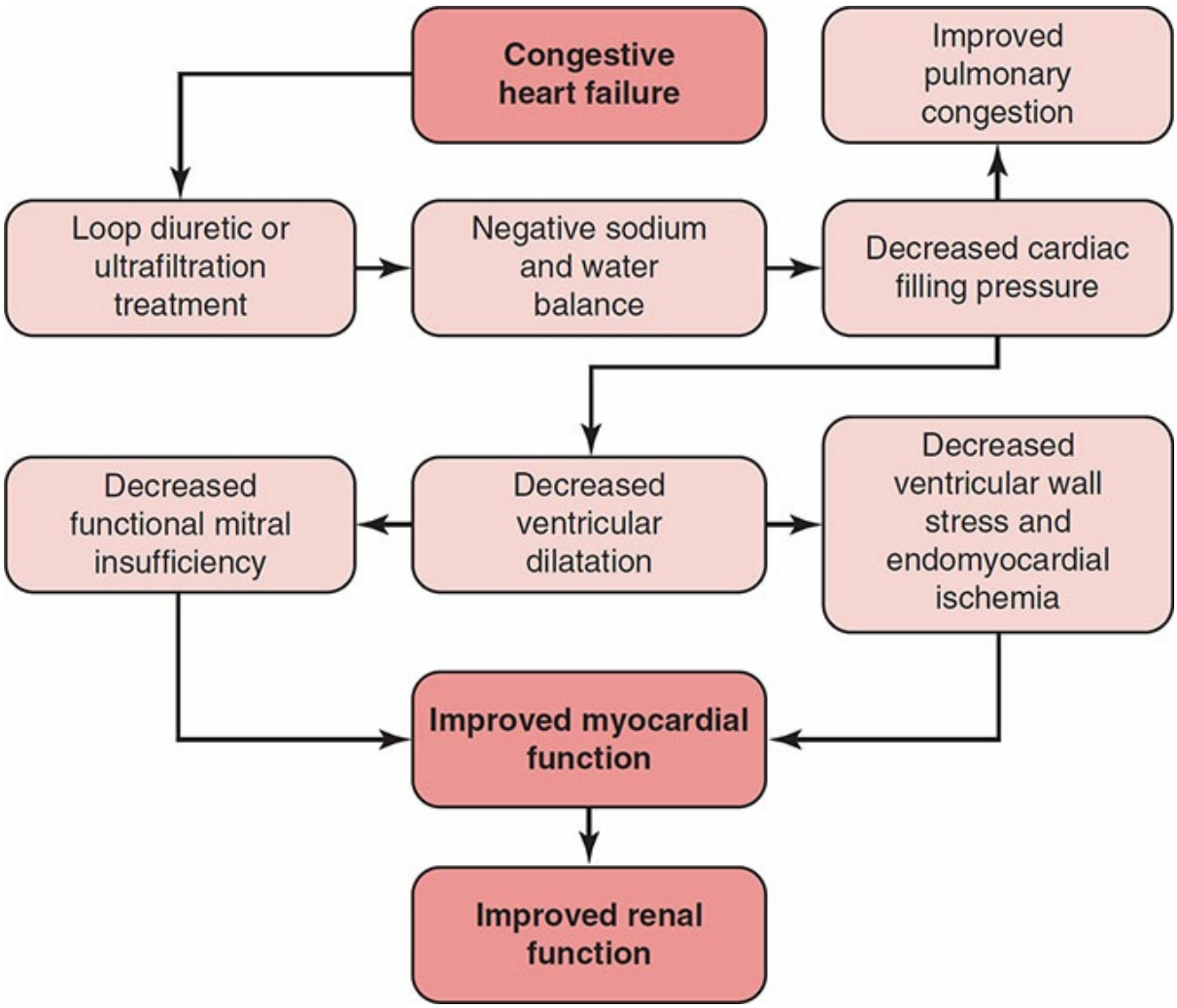

Figure 2–4 Mechanisms in congestive heart failure whereby negative sodium and water balance by loop diuretics or ultrafiltration therapy may improve myocardial and

renal function. (Reprinted from Schrier RW. Role of diminished renal function in cardiovascular mortality: marker or pathogenetic factor? *J Am Coll Cardiol*. 2006;47(1):1–8, with permission from Elsevier.)

The Nonosmotic Release of Arginine Vasopressin in Cardiac Failure

Plasma AVP is often elevated in patients with CHF and correlates in general with the clinical and hemodynamic severity of disease and the serum sodium level. Using a sensitive radioimmunoassay for AVP, Szatalowicz et al. (75) initially showed that plasma AVP was detectable in 30 of 37 patients with cardiac failure and hyponatremia. It was concluded that the nonosmotic AVP release in these patients was the result of baroreceptor stimulation secondary to diminished cardiac output because these patients had sufficient hyponatremia and hypo-osmolality, which would normally suppress maximally the osmotic release of AVP. Riegger et al. (76) also have reported that several patients with heart failure had inappropriately high plasma AVP levels. Cardiac output increased and plasma AVP levels normalized when two of these patients were treated with hemofiltration to remove excess body fluid. Other studies have also incriminated AVP in hyponatremic CHF patients (77,78). Taken together, these observations demonstrate enhanced nonosmotic AVP release in response to a decrease in cardiac output (i.e., arterial underfilling).

Renal Effects of Arginine Vasopressin

AVP, via stimulation of its renal or V_2 receptor subtype, enhances water reabsorption in the distal nephron, namely, the cortical and medullary collecting ducts. The evidence supporting a role for AVP in the water retention of heart failure comes from studies using selective peptide and nonpeptide antagonists of the V_2 receptor of AVP in several animal models of cardiac failure. For example, Ishikawa et al. (79) have assessed the antidiuretic effect of plasma AVP in a low-output model of cardiac failure secondary to vena caval constriction in rats. Plasma AVP concentrations were increased in these animals, and an antagonist of the antidiuretic effect of AVP reversed the defect in water excretion. An orally active nonpeptide V_2 receptor AVP antagonist, OPC-31260, was originally described in 1992 (80). The intravenous administration of OPC-31260 during a dose-ranging study in normal human subjects was shown to increase urine output to a similar extent as 20 mg of furosemide given

intravenously (81). Virtually simultaneous publications by Xu et al. (82) from our laboratory and Nielsen et al. (83) demonstrated the upregulation of aquaporin 2 (AQP2) water channels in coronary-ligated rats with CHF. The latter group also demonstrated that AQP1 and AQP3 were not upregulated in this CHF model and that increased trafficking of the AQP2 to the apical membrane occurred. Our group further showed that a V_2 vasopressin antagonist reversed the upregulation of the AQP2 protein in the renal cortex and medulla of the CHF rats (81). This effect of the nonosmotic release of AVP to cause water retention in cardiac failure recently has been associated with increased transcription of messenger RNA (mRNA) for the AVP preprohormone in the rat hypothalamus (84).

In a study by Bichet et al. (85), the effect of the ACEI captopril and the α_1-adrenergic blocker prazosin to reverse the abnormality in water retention in patients with class III and IV heart failure was examined. The resultant cardiac afterload reduction and increased cardiac output with either agent were associated with improved water excretion and significant suppression of AVP in response to an acute water load. A role of angiotensin II in modulating the effect of AVP in heart failure was unlikely because captopril and prazosin had divergent effects on the renin–angiotensin system; yet their effects to suppress plasma AVP and improve water excretion were comparable. In this regard, it is important to note that in this study by Bichet et al. (85), the average decrease in mean arterial pressure was 5 mm Hg, a decrement that is less than the 7% to 10% necessary to activate the nonosmotic release of AVP (86). Thus, these results are compatible with the suggestion that a decrease in stroke volume and cardiac output, rather than a fall in mean arterial pressure, may sometimes be the primary stimulus for the nonosmotic release of AVP in low-output cardiac failure. The association of improved cardiac output and water excretion during afterload reduction is compatible with unloading of high-pressure baroreceptors leading to increased AVP release.

The most recent advance relative to the nonosmotic release of AVP in CHF is the FDA approval of vasopressin receptor antagonists for clinical use in the United States. Conivaptan, a combined V_1 and V_2 receptor antagonist, has been approved for treatment of hyponatremia in cardiac failure. This antagonist can be used inhospital by intravenous administration for 4 days. The potential effect of the combined V_1 and V_2 antagonist properties in heart failure is shown in Figure 2-5 (74). Recently, the first orally active V_2 receptor antagonist, tolvaptan, has been approved for use in cardiac failure, cirrhosis, and the syndrome of inappropriate

antidiuretic hormone (SIADH) (87). In association with the increase in plasma sodium concentration in hyponatremic CHF patients, the self-reported SF12 demonstrated a significant improvement in mental status in these patients. There are other V_2 receptor antagonists in phase 3 trials. Taken together, these agents are known as *aquaretics* to emphasize that the resultant increase in solute-free water excretion occurs in the absence of a change in electrolyte excretion. This is the major difference with diuretics that increase urinary sodium chloride and other electrolyte excretion. These aquaretic agents can correct plasma sodium concentration in the absence of fluid restriction. In chronic hyponatremia, the correction of plasma sodium concentration with an aquaretic should not exceed 8 mmol over 8 hours or 10 to 12 mmol over 24 hours in order to avoid osmotic demyelination.

Altered renal hemodynamics may contribute to water retention in heart failure in addition to persistent AVP secretion. Decreased RBF and increased filtration fraction would be expected to increase proximal reabsorption of sodium and water, thereby diminishing fluid delivery to distal diluting segments. Increasing distal fluid delivery by administration of furosemide has improved the diluting ability of patients with heart failure (88).

In summary, activation of the sympathetic nervous system, the renin–angiotensin–aldosterone system, and the nonosmotic release of AVP by exerting direct (tubular) and indirect (hemodynamic) effects on the kidneys are implicated in the renal sodium and water retention of heart failure. These neuroendocrine mechanisms appear to be activated in response to arterial underfilling and suppressed by maneuvers that restore the integrity of the arterial circulation toward normal. In addition, the effects of these neurohormonal vasoconstrictor systems may be counterbalanced by endogenous vasodilatory and natriuretic hormones.

Natriuretic Peptides in Cardiac Failure

The natriuretic peptides, including ANP and brain natriuretic peptide (BNP), circulate at increased concentrations in patients with heart failure (89,90). These peptide hormones possess natriuretic, vasorelaxant, and renin-, aldosterone-, and sympatho-inhibiting properties (91). Both ANP and BNP appear to be released primarily from the heart in response to increased atrial or ventricular end-diastolic or transmural pressures. We demonstrated that increased ANP production rather than decreased metabolic clearance was the major factor contributing to the elevated

plasma ANP concentrations in a study of ANP kinetics in patients with cardiac failure (92). This finding is consistent with the observed increase in expression of both ANP and BNP mRNA in the cardiac ventricles of humans and animals with heart failure (93,94). BNP has been shown to reduce pulmonary capillary wedge pressure (PCWP) and increase cardiac index in acute CHF (95). In a coronary ligation model of heart failure in rats, the infusion of a monoclonal antibody shown to specifically block endogenous ANP in vivo caused a significant rise in right atrial pressure, left ventricular end-diastolic pressure, and systemic vascular resistance (96). Thus, natriuretic peptides appear to attenuate to some degree the arterial and venous vasoconstriction of heart failure.

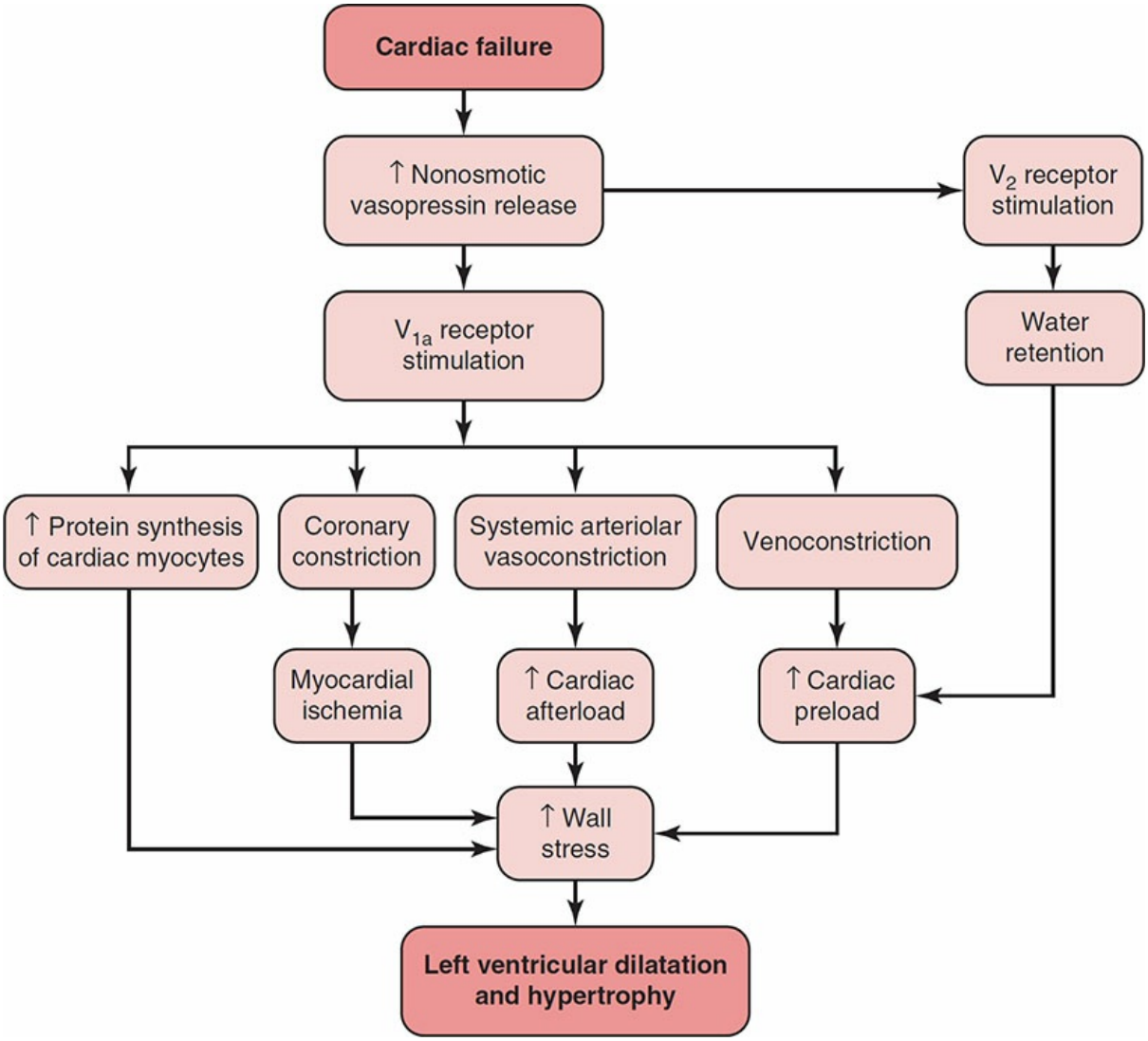

Figure 2–5 Pathways whereby vasopressin stimulation of V_2 and V_{1a} receptors can contribute to events that worsen cardiac function. (Reprinted from Schrier RW. Role of diminished renal function in cardiovascular mortality: marker or pathogenetic factor? *J Am Coll Cardiol*. 2006;47(1):1–8, with permission from Elsevier.)

Renal Effects of the Natriuretic Peptides

In normal humans, ANP and BNP increase GFR and urinary sodium excretion with no change or only a slight fall in RBF (97). These changes in renal hemodynamics are likely mediated by afferent arteriolar vasodilation with constriction of the efferent arterioles. However, in addition to increasing GFR and filtered sodium load as a mechanism of their natriuretic effect, ANP and BNP are specific inhibitors of sodium reabsorption in the collecting tubule (98). An important role for endogenous ANP in the renal sodium balance of heart failure has been demonstrated by Lee et al. (99). Similar decreases in cardiac output were induced in two groups of dogs by constriction of the TIVC or acute rapid ventricular pacing. Sodium retention paralleled the activation of the renin–angiotensin–aldosterone system in the TIVC constriction group. Atrial pressures and plasma ANP were not increased in this group of dogs. In comparison, the ventricular pacing group did not experience sodium retention or activation of the renin–angiotensin–aldosterone system. This group had similar reductions in cardiac output and arterial pressure as the TIVC constriction group but, unlike the TIVC constriction group, had increased atrial pressures and circulating endogenous ANP levels. In a third group, exogenous ANP was administered to TIVC constriction dogs to increase plasma ANP levels to those observed in the pacing model. The ANP infusion prevented the sodium retention and activation of the renin–angiotensin–aldosterone system.

Unfortunately, the administration of synthetic ANP to patients with low-output heart failure results in a much smaller increase in renal sodium excretion and less significant changes in renal hemodynamics compared with normal subjects (100). Like ANP, the natriuretic effect of BNP is blunted in rats with high-output heart failure produced by AV fistulas (101). In a trial of BNP, 127 patients with a PCWP of 18 mm Hg or higher and a cardiac index of 2.7 L/min/m^2 of body surface area or less were randomly assigned to double-blind treatment with placebo or BNP (nesiritide) infused at a rate of 0.015 or 0.030 μg/kg of body weight per minute for 6 hours (95). BNP significantly decreased PCWP and resulted in improvements in global clinical status in most patients (i.e., reduced dyspnea and fatigue). The most common side effect was dose-related hypotension, which was usually asymptomatic. Therefore, intravenous nesiritide may be useful for the short-term treatment of patients hospitalized with decompensated CHF (95). A recent retrospective report, however, demonstrated an increase in serum creatinine and mortality in

heart failure patients receiving BNP (102).

The possible mechanism of the relative renal resistance to natriuretic peptides in heart failure are the following:

1. Downregulation of renal ANP receptors
2. Secretion of inactive immunoreactive ANP
3. Enhanced renal neutral endopeptidase activity limiting the delivery of ANP to receptor sites
4. Hyperaldosteronism causing increased sodium reabsorption in the distal renal tubule
5. Diminished delivery of sodium to the distal renal tubule site of ANP action

A strong positive correlation between plasma ANP and urinary cGMP (the second messenger for the natriuretic effect of ANP and BNP in vivo) has been shown in sodium-retaining patients with heart failure (103). This observation supports the active biologic responsiveness of renal ANP receptors in heart failure and thus suggests that diminished distal tubular sodium delivery may explain the natriuretic peptide resistance observed in patients with cardiac failure. In cirrhosis, another edematous disorder associated with renal ANP resistance, increased distal tubular sodium delivery with mannitol has been shown to reverse the ANP resistance (104). Moreover, in heart failure, the administration of an angiotensin II receptor antagonist or furosemide, which is expected to increase distal tubular sodium delivery, also improves the renal response to ANP (64,105). Finally, studies in rats with experimental heart failure have demonstrated that renal denervation reverses the ANP resistance (106), an effect likely mediated by increased distal tubular sodium delivery. In Figure 2-6, the proposed role of diminished distal tubular sodium delivery in natriuretic peptide resistance and impaired aldosterone escape in states of arterial underfilling is shown.

Renal Prostaglandins in Cardiac Failure

Renal prostaglandins do not regulate renal sodium excretion or renal hemodynamics to any significant degree in normal subjects and intact animals. However, prostaglandin activity is increased in patients with heart failure and has been shown to correlate with the severity of disease as assessed by the degree of hyponatremia (107). Moreover, it has been well documented that the administration of a cyclooxygenase inhibitor in heart

failure patients may result in acute reversible renal failure, an effect proposed to result from inhibition of renal prostaglandins (108). An investigation in patients with moderate heart failure and a normal sodium intake demonstrated that the administration of acetylsalicylic acid in doses that decrease the synthesis of renal prostaglandin E_2 results in a significant reduction in urinary sodium excretion (109). These observations support a role for prostaglandins in attenuating the renal vasoconstriction and sodium retention in patients with heart failure.

PATHOGENESIS OF SODIUM AND WATER RETENTION IN CIRRHOSIS

Two earlier theories have attempted to explain the pathogenesis of sodium and water retention in cirrhosis (110,111). The classic "underfill hypothesis" suggested that ascites formation secondary to portal hypertension leads to decreased plasma volume, which secondarily increases renal sodium and water retention (110). However, results of animal studies have shown that sodium and water retention precedes ascites formation in cirrhotic animals, thus contradicting the hypothesis (111). Moreover, plasma volume is increased, not decreased, in cirrhosis. An alternative hypothesis was therefore proposed in which primary renal sodium and water retention occurs secondary to a hepatorenal reflex. This would lead to plasma volume expansion of both the venous and arterial compartments and cause overflow ascites (111). This "overfill hypothesis" of ascites formation in cirrhotic patients, however, did not explain the progressive stimulation of the neurohumoral profile as cirrhotic patients progress from compensated to decompensated with ascites to hepatorenal syndrome. Against this background, we have suggested a primary role for systemic arterial vasodilation for the initiation of renal sodium and water retention in cirrhosis (Fig. 2-2) (112,113). This theory encompasses the entire range of cirrhosis from compensated to decompensated to hepatorenal syndrome and explains the progressive increases in both plasma volume and neurohormonal activation that occur as cirrhosis worsens.

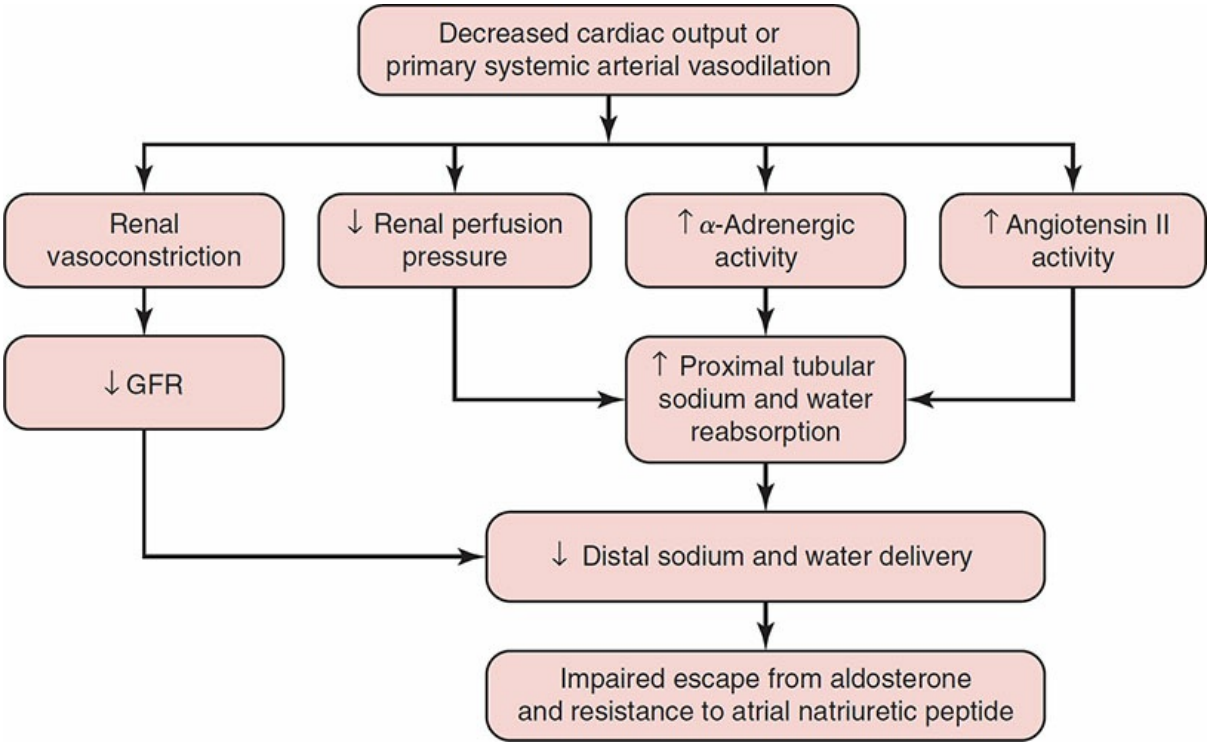

Figure 2–6 Proposed mechanism of natriuretic peptide resistance and impaired aldosterone escape in states of arterial underfilling. GFR, glomerular filtration rate. (Reprinted from Schrier RW. Water and sodium retention in edematous disorders: role of vasopressin and aldosterone. *Am J Med*. 2006;119(7 Suppl 1):S47–53, with permission from Elsevier.)

Systemic Arterial Vasodilation Hypothesis

Splanchnic arterial vasodilation occurs early in cirrhosis, and the resultant arterial underfilling stimulates sodium and water retention with plasma volume expansion before ascites formation. The normal plasma hormone concentrations in compensated cirrhotic patients are relatively increased for the degree of sodium and water retention and plasma volume expansion. The mediators of the early splanchnic vasodilation in cirrhosis may include the opening of existing shunts, activation of vasodilating hormones, and ultimately the development of collaterals. Vasodilation may occur at other sites including the skin, muscle, and lung as cirrhosis progresses. However, although the presence of splanchnic arterial vasodilation is well documented in experimental and human cirrhosis, the development of arterial vasodilation involving other vascular territories is less certain.

Increased synthesis and release of the potent vasodilator nitric oxide, perhaps owing to increased circulating levels of endotoxin in cirrhosis, have been proposed to account for the arterial vasodilation and

hyperdynamic circulation seen in cirrhotic patients (114–117). Although nitric oxide activity is difficult to assess in vivo, indirect evidence supports this hypothesis. For example, urinary cGMP, the second messenger of nitric oxide, is increased in patients with cirrhosis before the development of ascites and in some patients before an increase in circulating ANP concentrations (117). Markedly increased cGMP concentrations in aortic tissue from rats have been demonstrated in experimental cirrhosis (116). In these animals, aortic cGMP concentrations correlated inversely with arterial pressure ($r = -0.54$, $P < 0.0001$). Significantly, the chronic administration of the nitric oxide synthesis (NOS) inhibitor N^G-nitro-L-arginine-methyl-ester (L-NAME, 10 mg/kg/day for 7 days) induced a marked reduction in aortic cGMP concentration and an increase in arterial blood pressure in cirrhotic rats to similar levels obtained in L-NAME–treated control animals. This indicated that the high aortic cGMP content and decreased arterial blood pressure in cirrhotic rats were due to an increased NOS (116). Normalization of vascular nitric oxide production by chronic NOS inhibition corrects the systemic hemodynamic abnormalities in cirrhotic rats with ascites (117). Furthermore, chronic L-NAME treatment in drinking water for 10 days normalized mean arterial pressure, cardiac output, and systemic vascular resistance in these cirrhotic animals (118). The neurohumoral response in cirrhotic rats was also normalized as PRA, aldosterone, and AVP returned to control levels after 7 days of NOS inhibition (119). These hemodynamic and neurohumoral alterations during NOS inhibition were associated with profound reversal of the sodium and water retention in these cirrhotic rats. Moreover, Guarner et al. (115) have demonstrated elevated serum nitrite and nitrate levels—a crude index of in vivo nitric oxide generation—in 51 cirrhotic patients. Of note, in these patients, the elevated serum nitrite and nitrate levels significantly correlated with plasma endotoxin levels and decreased in response to a reduction in plasma endotoxin concentration following the administration of the antibiotic colistin (115). In addition, an enhanced sensitivity to mediators of endothelium-dependent vasodilation has been demonstrated in human cirrhosis (120). Taken together, these observations are compatible with the presence of nitric oxide–induced arterial vasodilation in cirrhosis. Endogenous opioids may also contribute to the peripheral vasodilation and renal sodium and water retention in cirrhosis, as the administration of opioid antagonists (e.g., naloxone or naltrexone) increased sodium and water excretion after water loading in cirrhotic subjects (121). Other factors that have been proposed to mediate the splanchnic vasodilation in cirrhosis include vasodilating prostaglandins,

glucagon, calcitonin gene–related peptide, platelet-activating factor, substance P, and vasoactive intestinal peptide; however, definitive proof is lacking for these potential medications. As with cardiac failure, pretreatment hyponatremia and high plasma concentrations of renin, norepinephrine, and aldosterone portend a poor prognosis in the cirrhotic patient. The highest plasma concentrations of these hormones and the lowest blood pressures occur as the decompensated cirrhotic patient with ascites progresses toward the hepatorenal syndrome.

Nephron Sites of Sodium Retention in Cirrhosis

There is indirect evidence for both enhanced proximal and distal tubular reabsorption in human cirrhotic subjects. The following findings support enhanced proximal tubular reabsorption in hepatic cirrhosis: (a) maneuvers that expand plasma volume and increase distal nephron delivery of fluid (i.e., head-out neck immersion and infusion of saline or mannitol) result in increased renal sodium excretion and solute-free water formation independent of changes in GFR (122); (b) in some water-loaded cirrhotic patients with ascites and minimal urine osmolalities, urine flow rates (an index of distal delivery of tubular fluid under these circumstances) are lower than in normal subjects (123); and (c) enhanced proximal reabsorption of tubular fluid has been found in micropuncture studies with chronic bile duct ligation (124).

Evidence for enhanced distal nephron sodium reabsorption is based on the following observations: (a) water-loaded patients with sodium retention and cirrhosis with minimal urine osmolalities often have urine flow rates comparable to normal controls (125); (b) water-loaded cirrhotic patients with minimal urine osmolalities have increased calculated distal fractional sodium reabsorption after receiving hypotonic saline infusions (125); (c) acetazolamide, a diuretic acting at the proximal tubule, produces a significant natriuresis in cirrhotic subjects only when there is concomitant distal nephron blockade of sodium reabsorption with ethacrynic acid (126); and (d) micropuncture studies in the dimethylnitrosamine and bile duct ligation models of cirrhosis demonstrate enhanced distal nephron sodium reabsorption (127,128).

In summary, clinical and experimental studies suggest that both proximal and distal nephron sites participate in enhanced renal tubular sodium reabsorption in cirrhosis. As in cardiac failure, neurohormonal activation appears to play a major role in the sodium and water retention of cirrhosis. The mechanisms responsible for enhanced sodium and water

reabsorption in cirrhosis are no doubt multifactorial. A decrease in GFR may not be observed in some sodium-retaining cirrhotic patients, suggesting that sodium retention can occur independently of a decrease in GFR. An increase in renal vascular resistance and filtration fraction often is seen in decompensated cirrhosis. Thus, peritubular physical forces (decreased hydrostatic pressure and increased oncotic pressure) may act to enhance proximal tubular sodium reabsorption in advanced cirrhosis.

The Sympathetic Nervous System in Cirrhosis

Elevated plasma levels of norepinephrine have been observed in cirrhotic patients with ascites. Plasma norepinephrine levels correlate positively with plasma AVP concentrations and PRA and negatively with urinary sodium excretion (122). Moreover, norepinephrine spillover rates in cirrhotic patients have been shown to be increased compared with normal controls, whereas norepinephrine clearance rates were comparable between the two groups (129). Floras et al. (130), using the technique of peroneal nerve microneurography to directly measure sympathetic nerve activity to muscle, also have demonstrated adrenergic activation in cirrhotic patients. Finally, Ring-Larsen et al. (131) have demonstrated normal hepatic norepinephrine clearances and increased renal norepinephrine release in cirrhotic patients. Taken together, these findings are compatible with the presence of systemic and renal adrenergic activation in cirrhosis.

These findings indicate that increased activity of the sympathetic nervous system and renal nerves may result in enhanced renal sodium reabsorption in cirrhosis. As mentioned, renal adrenergic stimulation has been shown to increase proximal tubular sodium reabsorption. In addition, a negative correlation between plasma norepinephrine and urinary sodium excretion has been shown in cirrhotic patients (132). Ring-Larsen et al. (133) have demonstrated an inverse correlation between plasma norepinephrine and RBF. Moreover, in the report from Floras et al. (130), muscle sympathetic nerve activity was inversely correlated with urinary sodium excretion.

Role of Aldosterone in Cirrhosis

In a study by Gregory et al. (134), 16 out of 21 cirrhotic patients exhibited disappearance of ascites with spironolactone treatment over 3 to 4 weeks; sometimes, a loop diuretic furosemide was added. Thus, the near-uniform

natriuretic response to spironolactone in cirrhotic patients, when given in adequate doses (100–400 mg/day), suggests that the increased levels of aldosterone contribute to the increased distal sodium reabsorption. Since exogenous aldosterone administration does not cause edema in normal subjects and absence of edema is a hallmark of primary hyperaldosteronism, the major problem in cirrhotic patients appears to be related to a failure to escape from the sodium-retaining effect of aldosterone as occurs in normal subjects. Aldosterone escape in normal subjects is associated with increased sodium delivery to the distal collecting duct site of aldosterone action. In cirrhosis, the increased neurohumoral activation, particularly angiotensin and α-adrenergic stimulation, enhances proximal tubular sodium reabsorption and diminishes distal sodium delivery. This sequence of events appears to be the main cause for the failure of cirrhotic patients to escape from the sodium-retaining effect of aldosterone. Because of the elevated endogenous plasma level of aldosterone, mineralocorticoid antagonists, such as spironolactone, need to be given in higher doses. Diuretic resistance in cirrhosis is therefore defined as absence of a natriuresis with daily spironolactone doses of 400 mg and furosemide of 160 mg. On this background, mineralocorticoid antagonists are established as the diuretic of first choice in cirrhotic patients, followed by a loop diuretic if necessary.

The Nonosmotic Release of Vasopressin in Cirrhosis

Hyponatremia with impaired ability to excrete a water load occurs in a substantial number of patients with cirrhosis of the liver, thereby demonstrating an impairment in urinary dilution in these patients (135,136). Decompensated cirrhotic patients with ascites and/or edema have an abnormal response to water administration, whereas cirrhotic patients without ascites or edema usually excrete water normally (136). There are two potential mechanisms for this inability to excrete solute-free water in decompensated cirrhotic patients with ascites: (a) a derangement in renal hemodynamics with decreased fluid delivery to the distal nephron; and (b) an extrarenal mechanism involving nonosmotic AVP release. Volume expansion maneuvers that improve distal fluid delivery of ascitic fluid (137,138), as well as head-out water immersion (122), improve urinary dilution and water excretion in cirrhosis. These maneuvers also increase central blood volume and could improve water excretion by suppressing baroreceptor-mediated nonosmotic AVP release.

Studies of patients with cirrhosis also implicate the nonosmotic release of AVP as a major factor responsible for water retention in cirrhosis. Bichet et al. (138) studied 26 cirrhotic patients who received a standard water load (20 mL/kg). Patients could be separated into two groups on the basis of their ability to excrete this water load: those able to excrete >80% of the water load in 5 hours ("excretors") and those unable to excrete a water load normally ("nonexcretors"). Nonexcretors had lower serum sodium concentrations and higher plasma AVP levels after the water load. These nonexcretors also were found to have higher pulse rates, lower plasma albumin concentrations, higher PRA and aldosterone concentrations, and higher plasma norepinephrine levels than normonatremic cirrhotic patients with normal water excretion (139). A greater increase in systemic arterial vasodilatation in the nonexcretors is supported by these studies. Thus, arterial underfilling may provide the nonosmotic stimulus for AVP release in hyponatremic cirrhotic patients. Enhancement of central blood volume by water immersion to the neck suppressed AVP release and improved, but did not normalize, water excretion in subsequent experiments (122). However, a comparable suppression of AVP with head-out water immersion and norepinephrine infusion normalized water excretion in decompensated cirrhotic patients (140). The increment in water excretion with this combined maneuver, which increases renal perfusion pressure, would be expected to increase distal fluid delivery.

Studies performed in rats made cirrhotic by exposure to carbon tetrachloride and phenobarbital supported AVP hypersecretion as the predominant mechanism of the impairment of water excretion because the administration of a V_2 vasopressin antagonist normalized water excretion in 9 of the 10 rats studied (141). Moreover, using the orally active nonpeptide V_2 receptor AVP antagonist OPC-31260, Tsuboi et al. (142) normalized the defect in solute-free water excretion in this animal model of cirrhosis. Additional experimental data supporting a primary role for AVP in the impaired water excretion in cirrhosis were reported by Fujita et al. (143). These investigators examined the effect of experimental cirrhosis on expression of the mRNA for the AVP-dependent collecting duct water channel, AQP2, in rats. Binding of AVP to the V_2 receptor initiates a chain of intracellular signaling events that leads to the insertion of AQP2 water channels into the apical membrane of collecting duct cells, thus rendering these cells permeable to water. In the cirrhotic rats studied by Fujita et al. (143), AQP2 mRNA was markedly increased as compared with control

animals. Moreover, an oral water load (30 mL/kg) did not reduce AQP2 mRNA expression, but the blockade of AVP action by the V_2 receptor AVP antagonist OPC-31260 significantly diminished its expression in the cirrhotic animals. Nonpeptide V_2 receptor antagonists have been shown to increase plasma sodium concentration in hyponatremic cirrhotic patients and improve urinary dilution (144,145).

Natriuretic Peptides in Cirrhosis

As with other edematous states associated with arterial underfilling, the neurohumoral responses to the systemic arterial vasodilation of cirrhosis are associated with factors that diminish distal sodium delivery. The impaired aldosterone escape (146) and resistance to ANP (147) that occur in cirrhosis, therefore, are most likely mediated by diminished distal sodium delivery to the collecting duct site of these hormonal actions. As with experimental cardiac failure, renal denervation has been shown to reverse the resistance to ANP in experimental cirrhosis (148). This finding supports a role of diminished distal sodium delivery in the ANP resistance. Moreover, Skorecki et al. (147) have demonstrated a normal increase in urinary cGMP but no natriuresis in some cirrhotic patients infused with ANP. Since cGMP is the secondary messenger of ANP, this finding supports the biologic responsiveness of renal ANP receptors in these patients. An increased distal sodium delivery with mannitol (as assessed by lithium clearance) has been shown to reverse resistance to exogenous ANP.

Renal Prostaglandins in Cirrhosis

Zambraski and Dunn (149) demonstrated that prostaglandins with vasodilator properties are necessary to maintain RBF and GFR in dogs with cirrhosis secondary to bile duct ligation. Similar conclusions about the importance of prostaglandins have been obtained in cirrhotic humans. Inhibition of prostaglandin synthesis in decompensated cirrhotic patients decreases RBF, GFR, sodium excretion, and solute-free water excretion and impairs the natriuretic response to diuretic agents (150,151). Infusion of prostaglandin has been shown to reverse the diminutions in RBF and GFR observed after prostaglandin inhibition in cirrhotic patients (151). Moreover, inhibition of prostaglandin synthesis may cause a syndrome that mimics the hepatorenal syndrome (150). Vasodilating renal prostaglandins may also play an important counterregulatory role in early or well-

compensated cirrhosis (152).

In summary, numerous afferent and efferent mechanisms are involved in the abnormal sodium and water excretion seen in patients with liver disease. These mechanisms appear to be initiated by arterial underfilling caused by primary systemic arterial vasodilation. The sympathetic nervous system, renin–angiotensin–aldosterone axis, and the nonosmotic release of AVP are the major effector components of this increased sodium and water reabsorption, which may also be modulated by the release of natriuretic peptides and renal prostaglandins.

PATHOGENESIS OF SODIUM AND WATER RETENTION IN THE NEPHROTIC SYNDROME

Two views of the pathogenesis of edema formation in the nephrotic syndrome are illustrated in Figure 2-7. According to the “underfill” theory, urinary loss of albumin occurs as a consequence of an increase in glomerular capillary permeability and results in hypoalbuminemia. This decline in serum albumin lowers intravascular colloid oncotic pressure, thereby increasing transudation of plasma from the intravascular to the interstitial space. It is this decrease in plasma volume that causes arterial underfilling and serves as the stimulus for renal sodium and water retention. Ultimately, the decrease in intravascular colloid oncotic pressure and the increase in interstitial hydrostatic pressure secondary to edema formation come into balance, and the edematous state stabilizes. Thus, the diminution in total plasma volume is the critical afferent stimulus in inducing renal sodium and water retention and should be observed in the initiating phase of formation. Several lines of evidence support this traditional underfill theory of edema formation in the nephrotic syndrome (153): (a) plasma volume may be modestly decreased in some nephrotic patients in the absence of diuretic therapy; (b) systemic arterial hypotension and diminished cardiac output, correctable by plasma volume expansion, have been observed in some patients with nephrotic syndrome; (c) some nephrotic patients have humoral markers of arterial underfilling such as elevated plasma levels of PRA, aldosterone, and catecholamines; and (d) head-out water immersion and intravascular infusion of albumin, maneuvers that increase plasma volume, may result in substantial increases in GFR and in fractional excretion of sodium chloride and water in these patients.

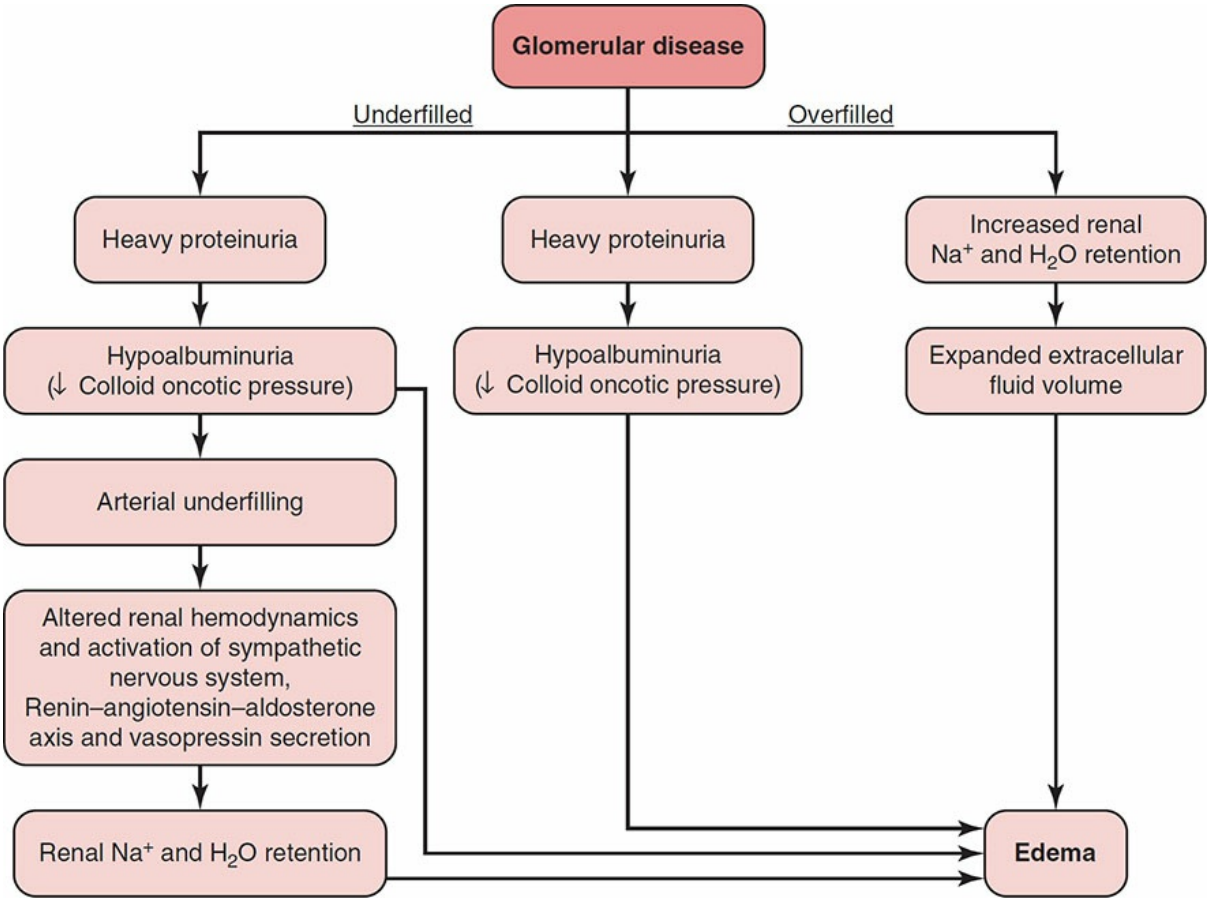

Figure 2–7 Proposed underfilled and overfilled mechanisms of sodium and water retention in the nephrotic syndrome. (Adapted from Bansal S, Lindenfeld J, Schrier RW. Sodium retention in cardiac failure and cirrhosis: potential role of natriuretic doses of mineralocorticoid antagonist? *Circ Heart Fail*. 2009;2(4):370–376.)

Usberti et al. (154) have described two groups of nephrotic syndrome patients distinguished on the basis of their plasma albumin concentrations. Patients in the first group had a plasma albumin concentration of <1.7 g/dL associated with low blood volumes and plasma ANP levels, elevated plasma angiotensin II concentrations, and increased proximal tubular reabsorption of sodium (determined by lithium clearance). In contrast, the second-group patients had a plasma albumin concentration >1.7 g/dL and exhibited normal blood volumes and plasma hormone concentrations. In all patients, blood volume was positively correlated with the plasma albumin concentration, and PRA was inversely correlated with both blood volume and plasma albumin concentration. Of note, GFR was not different between the first- and second-group patients (100 ± 25 mL/min vs. 101 ± 22 mL/min, P = NS), whereas urinary sodium excretion was substantially lower in the first-group patients (4.88 ± 5.53 mEq/4 h vs. 29.9 ± 9.3 mEq/4 h, $P < 0.001$). Moreover, the acute expansion of blood volume in the first-group patients normalized PRA, plasma angiotensin II and aldosterone

concentrations, fractional sodium excretion, and lithium clearance, whereas circulating ANP concentrations increased. Taken together, these observations support the traditional underfill view of the pathogenesis of edema formation in the nephrotic syndrome.

To further explore the state of arterial filling in patients with the nephrotic syndrome, sympathetic nervous system activity was evaluated in six edematous patients with the nephrotic syndrome of various parenchymal etiologies and in six normal control subjects (155). As mentioned, increased adrenergic activity occurs in states of arterial underfilling and may be the earliest sign. Sympathetic nervous system activity was assessed by determining plasma norepinephrine secretion and clearance rates using a whole-body steady-state radionuclide tracer method. Patients were withdrawn from all medications 7 days before study. Mean creatinine clearances and serum creatinine concentrations were normal in both the nephrotic syndrome patients and controls. However, the nephrotic syndrome patients exhibited significant hypoalbuminemia (2.0 ± 0.4 g/dL vs. 3.8 ± 0.1 g/dL, $P < 0.01$). The supine plasma norepinephrine levels were elevated in the patients with the nephrotic syndrome as compared with controls. More significantly, the secretion rate of norepinephrine was significantly increased in nephrotic patients, whereas the clearance rate of norepinephrine was similar in the two groups (Fig. 2-8). PRA and plasma aldosterone, AVP, and ANP concentrations were not different in nephrotic syndrome patients compared with controls. These observations indicate that the sympathetic nervous system is activated in patients with the nephrotic syndrome before a significant fall in GFR or a marked activation of either the renin–angiotensin–aldosterone system or the nonosmotic release of AVP. These data also support the presence of arterial underfilling in the nephrotic syndrome.

Several investigators, however, have challenged this traditional underfill model based on the following observations: (a) several studies of plasma and/or blood volume in edematous nephrotic patients have reported either normal or elevated values (156); (b) hypertension and low PRA, two indices suggesting volume expansion, have been reported in some patients with nephrotic syndrome (157); (c) hypoalbuminemia in animal studies as well as in patients with analbuminemia do not necessarily lead to edema formation (158); and (d) a low filtration fraction is often observed in patients with the nephrotic syndrome (159), in contrast to the increased filtration fraction usually associated with states of arterial underfilling. Thus, the “overfill” hypothesis has been proposed to account for nephrotic

edema formation in some patients. According to this view, the renal retention of sodium and water occurs as a primary intrarenal phenomenon independent of systemic factors. In this setting, renal sodium and water retention produces an expanded plasma volume, and the overfilled plasma volume then leaks into the interstitium and induces edema formation. The hypoalbuminemia and decreased plasma oncotic pressure serve to enhance the formation of edema.

A possible explanation for the variable volume and humoral results obtained in patients with the nephrotic syndrome is that the afferent stimulus may not be attributed to a single mechanism. Specifically, patients with the nephrotic syndrome are heterogeneous with regard to type of renal lesion, GFR, presence of underlying systemic disease, degree of hypoalbuminemia, and diuretic usage. In rat studies, aminonucleoside-induced nephrosis was characterized by a decreased plasma volume, as well as a well-maintained GFR, and edema could be prevented by adrenalectomy. In contrast, nephrotic syndrome induced by nephrotoxic serum was characterized by increased plasma volume and a very low GFR, and edema occurred independently of the adrenal glands. In this regard, the studies of Meltzer et al. (157) also are of note. In 1979, these investigators characterized a group of patients with the nephrotic syndrome associated with volume depletion and stimulation of the renin–angiotensin–aldosterone system and described a second group with low or normal PRA and aldosterone concentrations and hypervolemia. The "hypovolemic" group was characterized by minimal change disease and well-preserved GFRs. These patients fit nicely into the traditional underfill schema depicted in the left panel in Figure 2-7. The "hypervolemic" patients were characterized by having chronic glomerulopathy and reduced GFR (mean, 53 mL/min) in addition to suppressed plasma concentrations of renin and aldosterone, findings consistent with intrarenal mechanisms contributing to the renal sodium and water retention and thus the overfill theory.

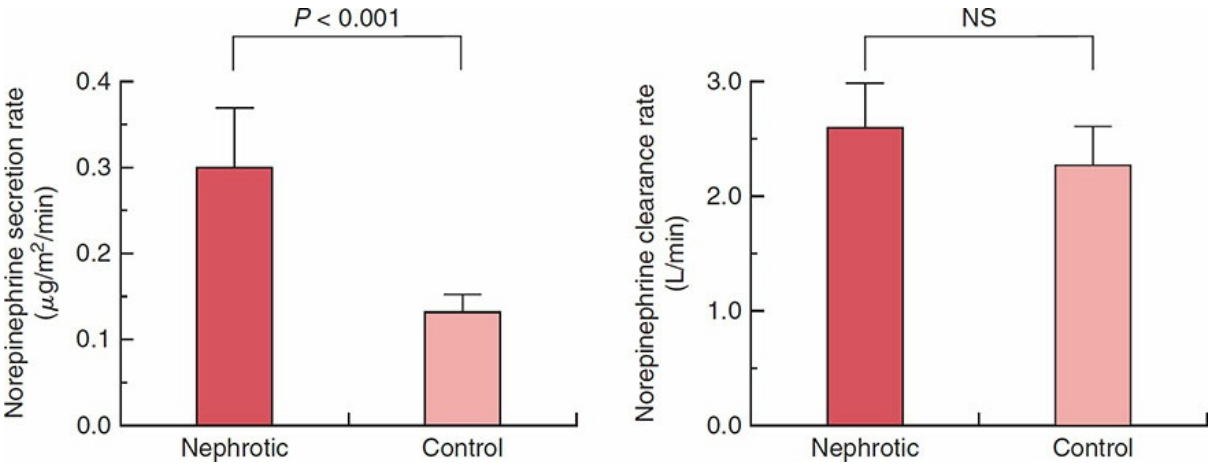

Figure 2–8 Plasma norepinephrine secretion and clearance rates in patients with the nephrotic syndrome and normal glomerular filtration rates and in normal control subjects. The findings of increased norepinephrine secretion and normal norepinephrine clearance in the nephrotic syndrome patients are consistent with early activation of the sympathetic nervous system in the nephrotic syndrome. (From Rahman SN, Abraham WT, Van Putten VJ, et al. Increased norepinephrine secretion in patients with the nephrotic syndrome and normal glomerular filtration rates: evidence for primary sympathetic activation. *Am J Nephrol*. 1993;13:266, with permission of S. Karger AG [Basel].)

Nephron Sites of Sodium Retention in the Nephrotic Syndrome

The nephron site of enhanced renal sodium retention in the nephrotic syndrome has been studied predominantly in animal models of glomerulonephritis. Bernard et al. (160) used micropuncture and clearance methodology to study the nephron site of increased sodium reabsorption in saline-loaded rats with autologous immune complex nephritis. These rats developed heavy proteinuria, hypoalbuminemia, and hypercholesterolemia. Etiopathologic examination of kidneys from these animals revealed slight thickening of basement membranes, uniform finely granular deposits of IgG and complement distributed along the basement membranes of all glomeruli, and electron-dense subepithelial deposits. These findings are similar to those observed in human idiopathic membranous nephropathy. Arterial blood pressure, hematocrit, GFR, and renal plasma flow were comparable in control and experimental animals. Proximal tubular sodium reabsorption was decreased in nephrotic rats as compared with controls (35% vs. 44%, $P < 0.05$). Absolute sodium reabsorption along the loop of Henle and in the distal convoluted tubule was comparable in nephrotic and control animals. Despite comparable sodium delivery to sites beyond the late distal convoluted tubule, the fractional excretion of sodium was significantly lower in nephrotic (2.2%)

than in control (4.0%) animals. From these results, the authors conclude that nephron sites beyond the late distal convoluted tubule are primarily responsible for the enhanced sodium reabsorption seen in this nephrotic model. Alternatively, it remains possible that enhanced sodium reabsorption by deep nephrons not accessible to micropuncture also could contribute to the diminished sodium excretion.

Different results were reported by Kuroda et al. (161) using a rat nephrotoxic serum model of nephrotic syndrome. Proteinuria, hypoalbuminemia, and hypercholesterolemia also occurred in these studies. Histologic examination of the kidneys revealed mild glomerular hypercellularity, widely dilated proximal tubules, diffuse glomerular linear immunofluorescence, and electron-dense subepithelial deposits. In contrast to the study by Bernard et al. (160), these animals were actively retaining sodium. In micropuncture studies, single-nephron GFR was decreased, and the percentage of filtered water reabsorbed before late proximal and distal tubular convolutions was increased in the nephrotic rats.

Two clearance studies have been undertaken in nephrotic patients in an attempt to clarify the nephron site of enhanced sodium reabsorption (162,163). Both of these studies were undertaken in patients with a wide variety of primary renal diseases and GFRs. Usberti et al. (163) measured tubular reabsorption of glucose in 21 patients with glomerulonephritis. Tubular glucose reabsorption was used as a marker for proximal tubular sodium reabsorption. The threshold for glucose reabsorption was reduced in 10 nephrotic patients, suggesting diminished proximal tubular reabsorption. A similar conclusion was reached by Grausz et al. (162) in studies undertaken in five nephrotic patients. Blockade of distal tubular nephron sites of sodium reabsorption with ethacrynic acid and chlorothiazide was used to assess proximal sodium reabsorption in these clearance studies. With this approach, proximal sodium reabsorption was found to be lower in nephrotic patients than in normal and cirrhotic patients. However, the more recent study of Usberti et al. (154) demonstrated increased proximal tubular sodium reabsorption—using the more precise technique of lithium clearance—in nephrotic syndrome patients with low albumin concentrations and blood volumes and elevated PRA.

In summary, it appears from experimental and clinical studies that distal nephron sites are primarily involved in the avid sodium retention of the nephrotic syndrome. However, it is likely that increased proximal tubular sodium reabsorption may also be operative in selected cases, depending on the nature of the underlying renal disease, the blood volume

status, and the phase of sodium retention.

Mechanisms of Enhanced Tubular Sodium Reabsorption

Several studies have been undertaken to identify the mechanism underlying the enhanced renal tubular sodium reabsorption in the nephrotic syndrome. Many nephrotic patients with a normal GFR avidly retain sodium, although a reduced GFR frequently is observed in nephrotic patients. Thus, factors in addition to a reduced filtered load of sodium are important in many nephrotic patients. Based on both experimental and clinical studies, however, nephrotic patients with the lowest GFR often demonstrate the greatest degree of sodium retention (157).

Peritubular capillary physical forces (oncotic and hydrostatic pressures) are believed to exert a modulating influence on renal sodium and water reabsorption. This influence is most likely exerted at the level of the proximal convoluted tubule. However, the low filtration fraction, high renal plasma flow, and normal renal vascular resistance frequently observed in nephrotic patients suggest that factors other than peritubular capillary physical forces are responsible for enhanced tubular sodium reabsorption.

The Renin–Angiotensin–Aldosterone System in the Nephrotic Syndrome

A potential role for the renin–angiotensin–aldosterone system in the pathogenesis of nephrotic sodium retention has been studied in detail. Two early experimental studies strongly supported a role for aldosterone in nephrotic edema (164,165). In rats made nephrotic with aminonucleoside, Tobian et al. (164) found an increase in juxtaglomerular cell granularity during sodium retention. Moreover, Kalant and collaborators (165) found that adrenalectomy prevented the sodium retention of aminonucleoside nephrosis. In one study, aminonucleoside was administered into one renal artery. Proteinuria, a reduced GFR, and sodium retention were observed only in the kidney that received aminonucleoside (166).

Several studies have measured components of the renin–angiotensin–aldosterone system in nephrotic humans (153). In general, studies have been carried out in heterogeneous patient populations at a variety of stages during the patients' illness. A wide range of values varying from very high to very low have been observed. However, PRA values tend to be highest in patients demonstrating characteristics of arterial underfilling and lowest

in overfilled patients.

Brown et al. (167) have undertaken clinical studies to examine the physiologic significance of activation of the renin–angiotensin–aldosterone system in sodium-retaining nephrotic patients. Eight of 16 patients had high PRA. Administration of the ACEI captopril to these eight patients did not induce a diuresis despite a significant reduction in plasma aldosterone to normal values. Mean arterial pressure, however, fell in these patients during converting enzyme inhibition. These results suggested that additional factors are responsible for the avid renal sodium retention even in nephrotic patients with high plasma aldosterone. Aldosterone antagonism studies are more definitive, however, because no fall in blood pressure secondary to diminished angiotensin II, as occurs with ACE inhibition, would occur. In this regard, we have demonstrated reversal of the positive sodium balance in patients with the nephrotic syndrome owing to a variety of glomerular diseases treated with the aldosterone antagonist spironolactone (168).

Natriuretic Peptides in the Nephrotic Syndrome

Plasma ANP and BNP concentrations may be elevated in humans and animals with the nephrotic syndrome (169,170); however, the hemodynamic and renal responses to exogenous ANP or BNP have been found to be blunted in experimental nephrosis (170) and in patients with the nephrotic syndrome (169). Perico and Remuzzi (171) have proposed tubular insensitivity to ANP as an initiating factor in the formation of edema in the nephrotic syndrome. According to their hypothesis, renal unresponsiveness to ANP results in distal tubular sodium and water retention with subsequent edema formation. Any ANP cellular resistance may result from a postreceptor, rather than receptor, mechanism, because urinary cGMP responds appropriately to ANP infusion in nephrotic animals (172). Alternatively, this blunted natriuretic response to ANP and BNP may be secondary to neurohormonal activation. The observed sympathetic activation in edematous patients with the nephrotic syndrome supports this possibility (155). Moreover, Koepke and DiBona (106) have shown that renal denervation, which is known to increase distal sodium delivery, reversed the blunted diuretic and natriuretic responses to ANP in a rat model of the nephrotic syndrome.

Other humoral factors (e.g., kinins and prostaglandins) may modulate renal sodium reabsorption in nephrotic patients. Specifically, inhibitors of prostaglandin synthesis have been reported to reduce GFR in patients with

the nephrotic syndrome and may precipitate renal insufficiency (173). Thus, prostaglandins may attenuate factors in nephrotic syndrome that decrease GFR and cause sodium retention.

Renal Water Retention in the Nephrotic Syndrome

The nephrotic syndrome is less frequently associated with hyponatremia, in contrast with the two previously described clinical edematous disorders, heart failure and cirrhosis. In fact, serum sodium concentration is usually normal unless it is influenced by vigorous diuretic measures or during an acute water load (174,175). Furthermore, high serum lipid levels may cause pseudohyponatremia in nephrotic patients unless serum sodium concentration is measured by a direct ion-specific electrode. Nevertheless, abnormal water excretion was clearly demonstrated by Gur et al. (176) in six nephrotic children because their solute-free water clearance during water loading was negative as compared with a positive value after remission of their disease. Head-out water immersion induced an increase in solute-free water clearance in patients with the nephrotic syndrome (177,178), and this improvement may have been secondary to the suppression of the nonosmotic release of AVP. Alternatively, water immersion might improve intrarenal hemodynamics, increase the amount of fluid delivered to the distal diluting nephron, and thereby improve water excretion. Plasma AVP concentrations have been found to be elevated in nephrotic subjects (175,179–181) and to correlate best with blood volume (181). Water immersion and hyperoncotic albumin infusion reduced plasma levels of AVP and induced a water diuresis in nephrotic patients (179–181). Studies by Shapiro et al. (182) have shown a close correlation between decrements in GFR and water excretion during an acute water load in patients with nephrotic syndrome. Therefore, analysis of these studies indicates that the impaired water excretion in nephrotic patients may be related both to intrarenal factors involving a fall in GFR and diminished distal fluid delivery and to extrarenal factors that primarily involve the nonosmotic release of AVP.

In summary, it appears that the effector mechanisms for sodium and water retention in the nephrotic syndrome may involve a fall in GFR and activation of the sympathetic nervous system, the renin–angiotensin–aldosterone system, and the nonosmotic release of AVP. Enhanced renal tubular sodium and water reabsorption observed in nephrotic patients also may involve a diminution in ANP sensitivity.

Treatment of Edematous Disorders

Given the preceding background discussion of the pathophysiology of sodium and water retention in the edematous disorders, the approach to the treatment of cardiac failure, liver disease, and the nephrotic syndrome is now considered. The general principles for such therapy are described in Table 2-2.

EVALUATION OF THE ADEQUACY OF TREATMENT OF THE PRIMARY DISEASE RESPONSIBLE FOR EDEMA

In cardiac failure, cirrhosis, and the nephrotic syndrome, the initiation of sodium and water retention involves the arterial underfilling caused by these diseases. Initial therapeutic attempts should be directed toward treatment of the primary disease. In low-output cardiac failure, the restoration of cardiac output to normal levels abolishes the arterial underfilling and thus the initiating event for renal sodium retention. The use of positive inotropic agents (e.g., digoxin) and afterload-reducing agents (e.g., ACEIs, ARB, arterial vasodilators) to improve cardiac output should be aggressively pursued in heart failure patients. This approach may alleviate the need for inhibiting tubular reabsorption with diuretics, a maneuver that may further decrease cardiac output and worsen the arterial underfilling. In this regard, it should be noted that the clinical practice guidelines for the treatment of heart failure from the Agency for Health Care Policy and Research recommend ACE inhibition as first-line therapy in nonedematous patients with heart failure (183). In the nephrotic syndrome, particularly of the nil disease or lipoid nephrosis variety, administration of corticosteroids may diminish or eliminate the proteinuria and thereby correct the hypoalbuminemia (184). In addition, treatment with ACEIs (185,186) or an ARB (187) has been shown to reduce the urinary protein loss associated with human nephrotic syndrome. In contrast, the administration of albumin solutions is of very little lasting value in the nephrotic syndrome because the concomitant increase in blood volume is associated with increased urinary clearance of albumin, and thus only a transient increase in plasma albumin concentration occurs. In extreme states of hypoalbuminemia, however, an infusion of albumin may be a lifesaving treatment for a hypotensive episode. Albumin solutions also may be of value for patients with cirrhosis, hypoalbuminemia, and edema, particularly when there is evidence of intravascular volume depletion, such as diminished central venous pressure and a fall in

orthostatic blood pressure. The administered albumin is excreted less readily in cirrhotic patients because they have no defect in glomerular capillary permeability and frequently have lower levels of GFR. However, a potential complication of such albumin infusions is the resulting increase in portal hypertension with increased bleeding from esophageal varices and the precipitation of hepatic encephalopathy because of the protein load. In some patients with acute alcoholic hepatitis accompanying cirrhosis, corticosteroid therapy may improve liver function in those with elevated bilirubin and prolonged prothrombin times.

Table 2–2 General Principles in the Treatment of Edematous Disorders

Evaluation of the adequacy of treatment of the primary disease responsible for edema
Evaluation of level of salt and water intake
Mobilization of edema: bed rest and supportive stockings
Evaluation of indications for use of diuretics Impaired respiratory function Incipient or overt pulmonary edema Elevated diaphragms with ascites, associated with incipient or overt atelectasis Impaired cardiovascular function secondary to fluid overload Excess fluid limiting physical activity and causing discomfort To avoid further sodium retention and yet allow the ingestion of palatable (sodium-containing) diet Cosmetic effect: marginal indication

EVALUATION OF THE LEVEL OF SODIUM AND WATER INTAKE

The level of sodium and water intake of edematous patients should be evaluated. However, it should be realized that although sodium restriction alone is effective in preventing further accumulation of edema, it may not induce a negative sodium balance. Patients who are edematous may be maximally retaining sodium (<10 mEq excretion per day). Thus, at best, “sodium-free” diets that contain 10 to 20 mEq of sodium merely prevent a further increase in positive sodium balance. The diuresis that may be observed in cardiac and cirrhotic patients who are hospitalized and placed on low-sodium diets may relate to the salutary consequences of bed rest on

cardiac output in the former and improvement in the primary liver disease in the latter, rather than to sodium restriction per se.

The level of fluid intake also must be assessed because most patients with edematous disorders have a defect in renal water excretion, as has been discussed, as well as in sodium excretion (Chapter 1 and the prior discussion). If the patient is hyponatremic, then the daily fluid intake should be adjusted to equal insensible losses (500–700 mL/day) plus daily urinary losses. However, severe fluid restriction often is difficult to accomplish in these patients because increased angiotensin II and baroreceptor activation may stimulate CNS thirst centers.

MOBILIZATION OF EDEMA

Bed rest alone may lead to a diuresis, particularly in patients with cardiac failure. Furthermore, patients who are resistant to diuretic agents administered on an outpatient basis may become responsive to the same or smaller doses of diuretic agents with hospitalization and bed rest. The use of professionally fit supportive stockings also may be of value in the mobilization of edema fluid. The mechanism of the supine position or supportive stockings, or both, in mobilizing edema fluid probably is related to the diminished peripheral venous pooling and thus to a more normal central arterial filling and renal perfusion. Finally, because upright posture in the normal person is associated with activation of the sympathetic nervous system and renin–angiotensin–aldosterone axis, the supine position may ameliorate to some extent overactivity of these neurohormonal vasoconstrictor mechanisms.

EVALUATION OF INDICATIONS FOR USE OF DIURETICS

If edema persists despite adequate treatment of the primary disease, then diuretics should be used only for definite indications (Table 2-2). The presence of edema alone is not an absolute indication for diuretic treatment, and any cosmetic value must be weighed against the potential deleterious effect of the drug. In general, the use of diuretic agents should be limited primarily to those situations in which impairment of respiratory or cardiac function, or both, or physical discomfort is secondary to fluid accumulation. An exception to this rule is the patient who will not restrict sodium intake; diuretics given to such patients may prevent edema accumulation despite dietary salt indiscretion. When patients find a low-salt diet unpalatable, diuretics can be used to allow them to include sodium

in their diet.

There are two cardinal rules to follow in estimating the optimal rate of diuresis once the decision is made to use diuretic agents to treat an edematous disorder. In general, the daily diuresis should approximate the rate of accumulation of the edema fluid. Thus, acute pulmonary edema necessitates induction of a rapid diuresis, whereas chronic heart failure is best treated with a more gradual diuresis. In either case, the rate of diuresis should be such that the rate of movement of interstitial fluid into the vascular compartment will not be exceeded to any large extent. If renal excretion does exceed the rate of mobilization of interstitial fluid, intravascular volume depletion and hypotension can result, even though ECF volume is still expanded. Careful clinical monitoring of intravascular volume (i.e., neck veins, orthostatic blood pressure, pulse, etc.), therefore, is extremely important, particularly during the induction of an acute diuresis. Intermittent diuretic therapy, such as alternate-day therapy, may be of value in avoiding intravascular volume depletion as well.

DIURETIC THERAPY

The judicious use of diuretic agents necessitates a knowledge of their site of action, potency, and side effects. In Table 2-3, the primary sites of action of the available diuretic agents are listed, but it should be emphasized that several of these diuretics also have secondary sites of action.

Site of Action of Diuretics

Filtration Diuretics

The so-called filtration diuretics are primarily aminophylline and glucocorticoids. In addition, plasma volume expansion or an increase in cardiac output secondary to use of cardiac glycosides or inotropic agents such as dopamine, dobutamine, or the phosphodiesterase inhibitors (e.g., amrinone, milrinone) may enhance GFR. Infusions of metaraminol and angiotensin II also have been shown to increase GFR in patients with cirrhosis, although these vasoconstrictor agents decrease filtration rates in normal subjects (123).

Table 2–3 Classification of Diuretics by Nephron Site of Action

Filtration Diuretics

Aminophylline
Glucocorticoids

Proximal Tubular Diuretics

Mannitol
Acetazolamide

Loop of Henle Diuretics

Ethacrynic acid
Furosemide
Bumetanide
Torsemide

Distal Tubular Diuretics

Potassium-losing
Thiazides
Chlorthalidone
Metolazone
Potassium-retaining

Triamterene
Spironolactone
Amiloride
Eplerenone

Collecting Duct Diuretics

Lithium
Demeclocycline
Vasopressin antagonists

Proximal Tubular Diuretics

Diuretics that act primarily to decrease proximal tubular sodium reabsorption include osmotic diuretics (e.g., mannitol) and carbonic anhydrase inhibitors (acetazolamide). The filtration and proximal tubular diuretics are not very effective when administered alone. Although the largest portion of glomerular filtrate is reabsorbed isosmotically in the proximal tubule (50%–70%), the distal nephron (particularly the ascending limb of the loop of Henle) has the capacity to increase its rate of sodium reabsorption significantly (188). Thus, an increase in glomerular filtration

or depression of proximal tubular reabsorption alone may not be associated with a significant diuresis because the increased distal sodium and fluid delivery may be reabsorbed at more distal nephron sites. Therefore, the filtration and proximal tubular diuretics are best used in conjunction with a diuretic that acts on the distal nephron, particularly when the patient has shown resistance to distally acting diuretics.

Loop of Henle Diuretics

The loop diuretics—ethacrynic acid, furosemide, bumetanide, and torsemide—are the most potent diuretic agents available. The inhibition by these agents of active sodium chloride transport in the medullary ascending limb of the loop of Henle generally exceeds the rate-limited sodium chloride reabsorption in the more distal nephron, and a maximal diuretic effect equivalent to 20% to 25% of the filtered load of sodium may be achieved.

The loop diuretics limit maximal renal diluting capacity because the reabsorption of tubular sodium chloride without water in the ascending limb is inhibited by these agents. It has been shown, however, that the administration of the loop diuretic furosemide may actually increase solute-free water excretion in edematous patients, who already have impaired diluting capacity (87). It has been proposed that the rapid rate of distal fluid delivery limits the osmotic equilibration between the collecting duct and interstitium because this diuretic-induced water diuresis is unaffected by exogenous AVP administration. Alternatively, the diuretic may interfere with the action of AVP on the collecting duct, particularly because thiazide diuretics increase and furosemide decreases urine osmolality in the presence of exogenous AVP at comparable solute excretion rates (189). Sodium chloride reabsorption in the ascending limb is also the major factor in the countercurrent concentrating mechanism that generates the hypertonicity in the medullary interstitium. Thus, loop diuretics also impair the renal capacity to concentrate the urine and conserve water. Finally, in contrast to other diuretics that may cause renal vasoconstriction, such as the thiazides, the loop diuretics cause renal vasodilatation, an effect that partially may contribute to their diuretic effect (190).

Distal Tubular Diuretics

The distal tubular diuretics can be classified into two groups: potassium-

losing and potassium-retaining diuretics. The thiazide diuretics, chlorthalidone and metolazone, have similar diuretic effects although they are chemically different. These distal tubular diuretics inhibit only urinary diluting capacity and not concentrating capacity because they decrease sodium reabsorption in the cortical, but not medullary, portion of the ascending limb as well as the distal convoluted tubule. As with the loop diuretics, the use of distal tubular diuretics, which also act proximal to the distal site of potassium secretion, is associated not only with a natriuresis but also with an increase in urinary potassium excretion. As discussed in Chapter 5, this effect of increased sodium delivery on potassium excretion has been shown to be linked to enhanced sodium reabsorption. Thus, an increase in distal sodium delivery may alter potassium excretion by modulating potassium secretion in the collecting duct (191).

The clinically available potassium-conserving diuretics include triamterene, amiloride, and the aldosterone antagonists spironolactone and eplerenone. Although the action of aldosterone antagonists is dependent on the presence of the adrenal cortex and circulating aldosterone, the ability of triamterene and amiloride to block potassium secretion is independent of adrenal function. The effect of amiloride appears to result from inhibition of sodium entry into the cell from luminal fluid by blocking the epithelial sodium channel. Frequently, these diuretics are not potent enough alone, but they may be used to avoid the potassium-losing effects of diuretics that act at more proximal nephron sites, such as the thiazide and loop diuretics.

Collecting Duct Diuretics

In contrast to all the diuretics mentioned, two diuretics act at the level of the collecting duct and induce a water diuresis rather than a natriuresis. These agents, demeclocycline and lithium, impair the ability of AVP to increase the water permeability of the renal collecting duct epithelium, thereby antagonizing the hydro-osmotic effect of AVP (192). These agents have been given only to hyponatremic, edematous patients because they induce a water diuresis. Both agents are capable of inducing significant adverse effects; however, one study demonstrated superior effect with less toxicity when demeclocycline was compared with lithium in the treatment of the syndrome of inappropriate AVP secretion (192). Nonetheless, demeclocycline has been shown to be nephrotoxic in hyponatremic cirrhotic patients and thus should be avoided in the presence of liver disease (193), including heart failure with hepatic hypoperfusion and

congestion. V_2 receptor AVP antagonists, which directly antagonize the renal effects of AVP, are now available (194). Conivaptan is a nonpeptide, combined V_1/V_2 receptor antagonist that has been approved to treat hyponatremia in euvolemic conditions (e.g., SIADH) and heart failure inhospital. The drug causes an increase in solute-free water excretion without an increase in electrolyte excretion. As noted earlier, conivaptan has been approved for intravenous use for a maximum of 4 days inhospital to increase plasma sodium concentration. The first orally active, nonpeptide V_2 antagonist, tolvaptan, has been approved to treat hyponatremia in euvolemic (e.g., SIADH) and hypervolemic states (heart failure and cirrhosis). Hyponatremia in heart failure and cirrhosis is a major risk factor for increased mortality in these conditions. The major side effects of these aquaretic agents include dry mouth, increased thirst, and polyuria. In chronic hyponatremia, the increase in plasma sodium concentration should not exceed 10 to 12 Eq/L over 24 hours because more rapid changes may cause osmotic demyelination (see Chapter 1).

Potency of Diuretics

All the thiazide-like drugs have reasonably comparable effects in optimal doses with the exception of metolazone, which is more potent than the others. The other thiazide diuretics differ from each other primarily in duration of action. Thiazide diuretics are probably the agent of choice when an oral agent of moderate potency is desired.

In optimal doses, the loop diuretics (ethacrynic acid, furosemide, bumetanide, and torsemide) are some six to eight times more potent than the thiazide diuretics. This greater potency is expected because several times more sodium chloride is reabsorbed in the loop of Henle than in the distal convoluted tubule. Because of their potency, the loop but not the thiazide diuretics are effective in patients with advanced renal failure (GFR <25 mL/min). Metolazone also has been shown to be effective in patients with a GFR of <25 mL/min and can enhance the effects of loop diuretics. The thiazide and loop diuretics both can be administered intravenously as well as orally.

Hemodynamic Effects of Diuretics

The hemodynamic actions of diuretics have been examined in normal humans, anephric subjects, patients with heart failure, and experimental

animals. In 1973, Dikshit et al. (195) reported the effects of intravenous furosemide (0.5–1.0 mg/kg) in 20 patients with left heart failure complicating acute myocardial infarction. These patients exhibited a marked decrease in left ventricular filling pressure, from 20.4 to 14.8 mm Hg, occurring between 5 and 15 minutes after furosemide administration. This effect anteceded the diuretic and natriuretic effect of the drug and was associated with a 52% increase in mean calf venous capacitance, thus demonstrating the venodilating effect of furosemide. This early venodilating effect of furosemide has been confirmed by other workers and also has been observed in normal subjects and experimental animals. The clinical importance of this early increase in venous capacitance and diminished left ventricular filling pressure resides in the resultant early beneficial effects of furosemide on acute pulmonary edema and explains the improved clinical symptoms of pulmonary congestion that may occur with furosemide before the onset of the drug's diuretic response. The acute venodilation associated with furosemide administration in these patients may be mediated by vasodilating prostaglandins because the administration of the prostaglandin synthetase inhibitor, indomethacin, has been shown to abolish the increase in venous capacitance initiated by furosemide in normal volunteers and anephric subjects consuming a low-sodium diet (196).

In contrast to the early venodilation observed in patients with acute left ventricular failure, intravenous furosemide has been shown to induce an acute vasoconstrictor response in patients with decompensated chronic class III and IV heart failure (196). In these patients with advanced heart failure, intravenous furosemide (1.3 mg/kg of body weight) caused a significant increase in mean arterial pressure and systemic vascular resistance, associated with a fall in stroke volume index and a rise in left ventricular filling pressure, 20 minutes after furosemide administration. This acute increase in cardiac afterload was associated with, and presumably resulted from, the accompanying rapid rise in circulating concentrations of three vasoconstrictor hormones, namely, plasma norepinephrine, angiotensin II, and AVP. In this regard, it is important to note that loop diuretics block NaCl transport at the macular densa that stimulates the renin–angiotensin system. Angiotensin II is also known to stimulate the sympathetic nervous system. Thus, it is clear that the acute vascular effects that occur with intravenous furosemide are determined, at least in part, by whether the patient has acute (197) versus chronic heart failure (198). With chronic treatment, however, diuretic therapy results in favorable effects on both cardiac preload and afterload, which may result

in an improvement in left ventricular function.

Neurohormonal Effects of Diuretics

The acute intravenous administration of furosemide with the resultant diuresis and natriuresis may be associated with activation of the sympathetic nervous system, renin–angiotensin–aldosterone system, and nonosmotic release of AVP in patients with acute heart failure. Likewise, chronic oral diuretic therapy (furosemide, 80–240 mg/day for 8 days) has been shown to increase plasma renin, angiotensin II, and aldosterone concentrations in chronic heart failure patients (198). Moreover, Bayliss et al. (199) have demonstrated a similar activation of the renin–angiotensin–aldosterone system during the chronic administration of oral furosemide, 40 mg/day, plus amiloride, 5 mg/day, for 30 days to patients presenting with decompensated heart failure manifest by pulmonary and/or peripheral edema.

In addition to the effect of diuretics to increase renal renin release, the diminished concentrations of natriuretic peptides that occur in association with chronic diuretic administration also may explain the further activation of the renin–angiotensin–aldosterone system because natriuretic peptides are known to suppress plasma renin and aldosterone synthesis and release. Support for this hypothesis may be found in studies performed in animal models of heart failure. Fett et al. (200) have studied the endocrine and renal effects of intravenous furosemide (1.7 mg/kg) in an animal model of acute low-output heart failure owing to rapid right ventricular pacing at 250 beats/min for 3 hours. In this model, 2 hours after furosemide, there was a fall in plasma ANP, RBF, and GFR as the renin–angiotensin–aldosterone system was activated. The authors then examined the effects of an exogenous infusion of ANP, sufficient to prevent the furosemide-induced fall in the elevated endogenous plasma ANP, in the same experimental model. Maintenance of plasma ANP concentrations was associated with an enhanced natriuretic response to furosemide at 1 hour (182 μEq/min vs. 440 μEq/min, $P < 0.05$) and at 2 hours (72 μEq/min vs. 180 μEq/min, $P < 0.05$), associated with suppression of plasma aldosterone and maintenance of GFR.

Intermittent versus Continuous Intravenous Diuretic Therapy for Decompensated Edematous States

Kaojarern et al. (201) have suggested the time course of delivery of such

diuretics as furosemide into the urine as an independent predictor of overall response. This observation led to the concept of a "maximally efficient excretion rate" for furosemide (201). In this regard, it is possible that a continuous infusion of furosemide or similar diuretic at a dose that constantly maintains the most efficient urinary diuretic excretion rate may be superior to intermittent intravenous diuretic administration. Studies in heart failure patients support this hypothesis. Lahav et al. (202) performed a prospective, randomized, crossover trial comparing intermittent intravenous furosemide administration (30–40 mg/8 hour for 48 hours) with a continuous furosemide infusion following a single loading dose (2.5–3.3 mg/hour for 48 hours after a 30- to 40-mg loading dose) in nine patients with advanced heart failure refractory to conventional oral therapy. Total doses of furosemide administered were equivalent in the two groups. The continuous infusion of furosemide produced greater diuresis and natriuresis compared with intermittent furosemide administration in all patients. Similar results have been obtained with furosemide or bumetanide in normal volunteers and patients with advanced renal dysfunction (203). These results suggest that the continuous infusion of a loop diuretic may be the preferred method for intravenous diuretic therapy in patients with decompensated disease or "diuretic resistance" (see the following). Torsemide oral bioavailability may be better and more consistent than other loop diuretics (204).

Side Effects and Complications of Diuretic Therapy

The most common complications of diuretic therapy are volume and potassium depletion (Table 2-4). The thiazide and loop diuretics are most commonly associated with these complications. Volume depletion can be profound and may be associated with symptoms of cerebral or coronary insufficiency, particularly in the elderly. Diminished renal perfusion also may occur, as evidenced by a rise in blood urea nitrogen and serum creatinine concentrations.

Table 2–4 Complications of Diuretic Therapy

Metabolic Complications
Volume depletion and azotemia
Hypokalemia and hyperkalemia
Hyponatremia

Acidosis and alkalosis
Carbohydrate intolerance
Hypomagnesemia
Hypocalcemia and hypercalcemia
Hyperuricemia

Hypersensitivity

Rash
Interstitial nephritis
Pancreatitis
Hematologic disorders

Miscellaneous

Deafness
Gastrointestinal symptoms

A high-potassium diet (e.g., oranges, apricots, bananas) is frequently sufficient to avoid diuretic-induced hypokalemia. However, potassium chloride supplements or potassium-retaining diuretics may be necessary to avoid this complication in many patients treated with moderate to high doses of loop and/or thiazide-type diuretics and metolazone. It is important to note that potassium supplements and potassium-retaining diuretics should only be administered simultaneously under very close supervision because of the potential danger of fatal hyperkalemia. This is also true for the combination of potassium supplements or potassium-retaining diuretics and ACEIs or ARBs, which inhibit aldosterone and thus promote potassium retention. Spironolactone has been shown to induce or worsen renal tubular acidosis in some cirrhotic patients (205). Even more careful monitoring of serum potassium concentrations is necessary during diuretic therapy for patients receiving cardiac glycosides because either hypokalemia or hyperkalemia are known to stimulate or exacerbate arrhythmias associated with digoxin excess.

Hyponatremia may result from the impaired water excretion associated with the primary edematous disorder, from the ability of the diuretic to impair urinary diluting capacity, or from a combination thereof. In either case, if diuretic therapy is indicated, any symptomatic hyponatremia associated with edematous states is better treated by water restriction than by cessation of diuretic therapy. If ineffective, the orally active V_2 antagonist, tolvaptan, may be useful. Metabolic acidosis is a complication of the use of carbonic anhydrase inhibition because these agents block

hydrogen ion secretion. The use of thiazide and loop diuretics may be associated with metabolic alkalosis. This is predominantly owing to the excretion of sodium, chloride, and potassium without bicarbonate, which leads to a rise in serum bicarbonate concentration.

The complication of carbohydrate intolerance has been observed with both the thiazide and loop diuretics and may be related to potassium depletion. Hypokalemia is known to blunt the insulin response to a carbohydrate load, and this mechanism accounts at least in part for the carbohydrate intolerance. Patients most affected by this complication are probably those with diabetes mellitus or those predisposed to it.

Hyperuricemia may occur with most diuretics but has been reported most widely with thiazide diuretics or furosemide therapy. The primary cause of the hyperuricemia is a reduced urine clearance, which has been attributed to the enhanced tubular sodium reabsorption associated with volume depletion because urate reabsorption in the proximal tubule parallels the rate of tubular sodium reabsorption.

Hypercalcemia has also been described in conjunction with thiazides given to normal subjects, hyperparathyroid subjects, and hypoparathyroid subjects treated with vitamin D (206). The negative sodium balance and positive calcium balance associated with thiazide treatment seem at least partially responsible for the hypercalcemic effect. An interrelationship between parathyroid hormone and thiazide diuretics has also been demonstrated. Because of their hypocalciuric effect, thiazide diuretics may be used in the treatment of the idiopathic hypercalciuria that afflicts some patients with renal calculi. This may be associated with an effect on Na^+/Cl^- cotransporter to enhance Na^+/Ca_2^+ exchange (207). In contrast, furosemide increases calcium excretion and therefore has been used in conjunction with saline infusions to treat hypercalcemia. Because of this hypocalcemic effect, furosemide may induce symptoms of tetany in patients with borderline hypoparathyroidism (208).

Hypersensitivity reactions causing an interstitial nephritis may occur in association with thiazide diuretics or furosemide. Acute renal failure may occur when nonsteroidal anti-inflammatory drugs (NSAIDs) and triamterene are administered simultaneously (209). Skin rashes and hematologic disorders are other manifestations of hypersensitivity reactions that have been observed with diuretic therapy. A Schönlein–Henoch type of purpuric lesion of the lower extremities has been seen during treatment with ethacrynic acid (210). The diuretic agent should be discontinued in the presence of any signs of hypersensitivity reactions similar to serum sickness. Acute pancreatitis also has been observed in

association with thiazide administration. Deafness, which is generally reversible on cessation of the diuretic administration, has been reported both with ethacrynic acid and with furosemide; in occasional cases, however, diuretic-induced deafness has been irreversible. Generally, this has occurred in patients with renal disease receiving acute bolus administration. Thus, the dose of loop diuretics should be given over 20 to 30 minutes when administered intravenously. Gastrointestinal disturbances may occur with any of the diuretic agents.

Causes of Diuretic Resistance

Resistance to diuretic therapy is most often owing to incomplete treatment of the primary disorder, continuation of a high sodium intake, or patient noncompliance. Inadequate diuretic dose or dosing regimen and route of administration may also be implicated in some cases. For example, given the 6-hour duration of action of oral furosemide, once-daily administration of this agent will be inadequate for most patients. As noted, in decompensated patients, continuous intravenous diuretic therapy may be superior to intermittent dosing regimens. Volume depletion is the most common cause of diuretic resistance once these above factors have been excluded. Because the most frequently used diuretics act at sites in the loop of Henle or distal convoluted tubule, their action is dependent on adequate delivery of sodium to these sites. Thus, diuretic-induced volume depletion with attendant decreases in GFR and increases in proximal tubular sodium reabsorption impairs the response to diuretics acting in the distal nephron. Because most diuretic agents exert their diuretic effect from the luminal side of tubular cells (as opposed to the contraluminal or peritubular capillary side), the delivery of the diuretic agent to its site of action in the nephron may also be diminished during volume depletion and decreased RBF. In this regard, it should be noted that triamterene may block the tubular secretion of furosemide, and this combination of diuretics should be avoided.

Diuretic-induced further activation of the sympathetic nervous and renin–angiotensin–aldosterone systems with a concomitant decrease in circulating plasma ANP may also contribute to the development of diuretic resistance because increased renal nerve activity and angiotensin II may enhance proximal tubular sodium reabsorption, thereby obscuring the beneficial effect of a diuretic that acts in the distal nephron. This mechanism provides the rationale for combination therapy with a diuretic and neurohormonal antagonist, such as an ACEI, in edematous states.

Volume depletion and diuretic-induced renin release also increase aldosterone secretion. The distal tubular effect of aldosterone may blunt the natriuretic and enhance the kaliuretic response to diuretics. Avoidance of diuretic-induced volume depletion can be obtained best by initiating diuretic therapy with one of the diuretic agents of lower potency. Subsequently, the dose may be carefully titrated upward or more potent diuretics added while the patient's weight and orthostatic pulse and blood pressure changes are being monitored. The intermittent use of diuretics may help to avoid intravascular volume depletion. Finally, aldosterone antagonists in combination with more proximally acting diuretics may help to promote a diuresis in patients with "resistance" who do not appear to be profoundly volume depleted. Aldosterone has also been shown to increase the NaCl cotransporter that could contribute to diuretic resistance (211). The role of diminished distal delivery and secondary hyperaldosteronism in diuretic resistance is shown in Figure 2-9.

The loop diuretics and thiazides are effective in the presence of acid–base disturbances. The diuretic effect of carbonic anhydrase inhibitors is blunted by the presence of respiratory or metabolic acidosis, possibly because of the excess of intracellular hydrogen ions even in the presence of carbonic anhydrase inhibition. Finally, the NSAIDs appear capable of attenuating the action of several diuretic agents (207).

USE OF DIURETICS IN SPECIFIC EDEMATOUS STATES

Cardiac Failure

It is the heart and not the kidney that fails in heart failure. The response of the kidney can be viewed as a normal compensatory attempt to restore arterial circulatory integrity. However, the increased renal sodium and water retention with heart failure results in increased venous return, further stretching of the diseased myocardium, pulmonary congestion, increasing renal venous pressure, and ultimately increased capillary filtration of fluid with peripheral and pulmonary edema. One form of therapy in chronic heart failure is to increase the contractile force of the heart with a cardiac glycoside, which has been shown to decrease the frequency of hospitalization but not alter mortality. The chronic use of other inotropic agents, however, has been shown to decrease survival, perhaps secondarily to arrhythmias and sudden death.

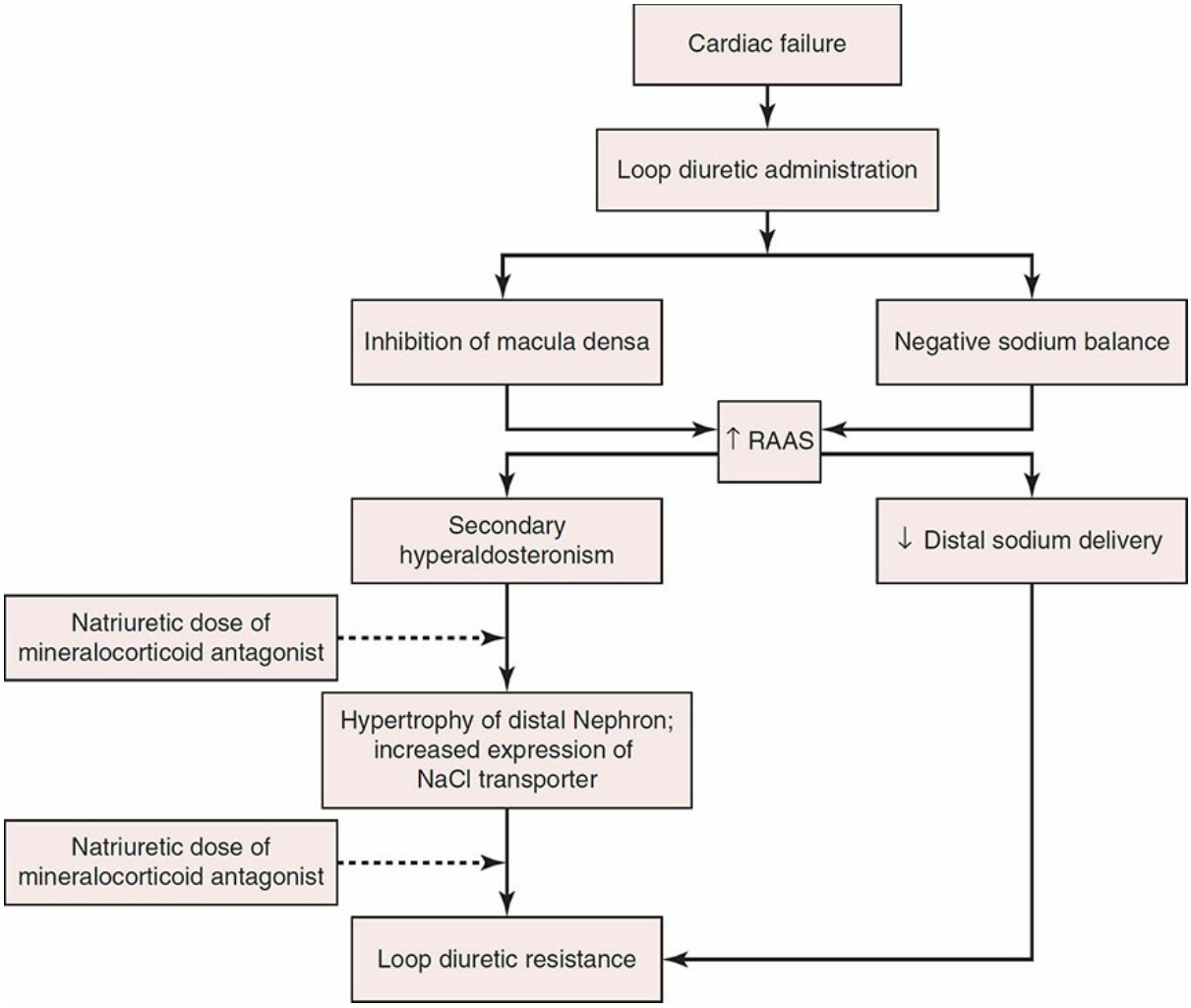

Figure 2–9 Mechanisms of diuretic resistance in heart failure. (From Bansal S, Lindenfeld J, Schrier RW. Sodium retention in heart failure and cirrhosis: potential role of natriuretic doses of mineralocorticoid antagonist? *Circ Heart Fail*. 2009;2(4):370–376, with permission from Wolters Kluwer Health, Inc.)

In addition to the effect of agents directly influencing the contractile state of the myocardium, it is important to note that the myocardial contractile state is also related to preload (venous return to the heart) and afterload (impedance to left ventricular outflow). Diuretic therapy diminishes preload by reducing venous return. This reduction in preload can reduce left ventricular filling pressure and thus alleviate some of the congestive symptoms of heart failure. Afterload reduction by systemic vasodilator therapy (parenteral nitroprusside or oral hydralazine, ACEIs, ARB, or prazosin) potentially results in improved ventricular function. The combined reduction in both preload and afterload induced by diuretics and vasodilators also may result in a favorable effect on cardiac performance.

In actuality, diuretics alone improve both congestive symptoms and exercise tolerance of patients with chronic heart failure (208–212). These

favorable effects may occur at the expense of a reduced cardiac output, however (210). Thus, diuretics should be used cautiously in patients with chronic heart failure. ACEIs alone are often effective in relieving symptoms in mild heart failure without substantial edema. However, this form of therapy may be inadequate, and diuretics and digoxin may be needed in more severe degrees of heart failure. Again, it is important to be aware of diuretic-induced decreases in serum potassium and magnesium concentrations that predispose to digitalis toxicity and cardiac arrhythmias when treating the patient with heart failure.

Specific recommendations after ACE inhibition or ARB for the use of diuretics in heart failure are as follows:

1. Start with a loop or thiazide-type diuretic, depending on the severity of the heart failure. For severe heart failure with substantial volume overload (i.e., overt pulmonary and/or peripheral edema), use a loop diuretic, given the greater potency of this class of agents. Thiazides are usually adequate in patients with mild heart failure.
2. Add a thiazide diuretic or metolazone if a loop diuretic given twice daily in doses equivalent to furosemide, 240 mg/day, is inadequate for diuresis. This combination generally results in a synergistic effect on salt and water excretion. *Note*: This combination also results in a synergistic effect on renal potassium excretion; therefore, anticipate an increase in the requirement for supplemental potassium to avoid hypokalemia.
3. A potassium-retaining diuretic may be added in order to spare potassium or enhance diuresis. However, as mentioned, do not combine triamterene with furosemide because triamterene blocks the tubular secretion of furosemide, thus inhibiting furosemide effect.
4. The goal of therapy is the resolution of signs and symptoms caused by pulmonary and/or peripheral edema.

In Figure 2-4, the potential mechanisms whereby judicious fluid removal by diuretics or ultrafiltration can improve cardiac function are shown. Nonnatriuretic doses of spironolactone have been shown to improve survival in cardiac failure, presumably via nonrenal effects on vascular and cardiac fibrosis. Natriuretic doses (>50 mg/day) of spironolactone have not been routinely used in heart failure patients, presumably because of the potential for causing hyperkalemia in the presence of ACE inhibition or ARB.

Cirrhosis

Some authors suggested that diuretic therapy is associated with substantial risk of adverse effects for the cirrhotic patient (213). The most feared complication is the induction of azotemia. Often this is the result of overzealous use of diuretics. Shear et al. (214) have demonstrated that the maximum rate of absorption of ascites from the peritoneum is 900 mL/day and is usually much less. A more rapid rate of diuresis (i.e., >1 L/day of negative fluid balance) occurs only at the expense of more easily mobilized peripheral edema fluid or diminished plasma volume. Hence, a profound diuresis may be associated with deterioration in renal function. Fortunately, such diuretic-induced azotemia is usually reversible; however, diuretics have been shown to precipitate hepatorenal syndrome in some cases. Alterations in serum potassium concentration are often encountered during diuretic treatment of the cirrhotic patient with ascites. Because secondary hyperaldosteronism and total body potassium depletion are frequently associated with cirrhosis (215), any diuretic that acts proximally to the distal potassium secretory site may cause profound hypokalemia unless it is accompanied by a potassium-sparing diuretic. Because of the frequently observed temporal relationship between diuretic therapy and induction of hepatic encephalopathy, Gabuzda and Hall (216) have postulated that the enhanced renal ammonia production of hypokalemia may contribute to the encephalopathy. In view of these potential hazards in the use of diuretics for the cirrhotic patient, the following general principles are recommended in the treatment of ascites:

1. Daily body weight and careful clinical and biochemical monitoring is mandatory.
2. Ascertaining that liver and renal functions are stable before instituting diuretic therapy.
3. Mobilizing ascites and edema with an initial period of bed rest and restricting dietary sodium before instituting diuretic therapy because conservative therapy alone may result in a diuresis in 5% to 15% of cirrhotic patients.
4. Aiming for a daily weight loss of 1 to 2 lb in patients with ascites with peripheral edema and 0.5 to 1 lb in patients with ascites but without peripheral edema.
5. Maintaining the end point of therapy as maximum patient comfort with a minimum of drug-induced complications. Occasionally, this may require slight liberalization of sodium intake at the expense of increased usage of diuretics and maintenance of some residual ascites

in selected patients.

The suggested regimen for diuretic therapy of ascites is as follows:

1. Restrict sodium (20–40 mEq/day).
2. If there is no diuresis in 3 to 4 days, add spironolactone initially (100 mg/day with increases every 3–5 days until natriuresis occurs as assessed by urinary sodium concentration). This approach results in a diuresis in 40% to 60% of patients.

Table 2–5 Therapy of Nephrotic Edema

Treatment of primary disorder
Conservative methods of therapy
Dietary (protein supplementation, salt restriction, water restriction)
Physical (recumbency, lower-extremity elevation)
Diuretic therapy
Pharmacologic agents
Albumin infusions
Water immersion
Miscellaneous (angiotensin-converting enzyme inhibition, increase protein intake)

3. If there is no diuresis with 400 mg of spironolactone per day, add hydrochlorothiazide (50–200 mg/day) or furosemide (20–80 mg/day). Diuretic resistance in cirrhosis has been defined as lack of response to 400 mg/day spironolactone and furosemide 160 mg/day.
4. Increasing doses of furosemide may be used if no diuresis is observed on this regimen after reassessment of dietary intake and hepatic and renal function show no deterioration.

It is of note that using a similar protocol, Gregory et al. (135) have documented that diuretic therapy can safely and efficaciously be given to the cirrhotic patient. Recently, numerous investigators have shown that repeated large-volume paracentesis (4–6 L/day) is a fast, effective, and safe therapy for ascites in patients with cirrhosis (216–222). The subsequent administration of diuretics avoids reaccumulation of ascites in the patients responding to these drugs. Of interest, contrary to the traditional concept of the potential danger of rapid and large paracentesis, the mobilization of ascites by paracentesis associated with intravenous

albumin (8 g/L of ascites removed) did not alter renal function or systemic hemodynamics, the latter being estimated either directly (by measuring plasma volume, cardiac output, or peripheral resistance) or indirectly (by measuring PRA, plasma norepinephrine, and plasma AVP concentration).

Nephrotic Syndrome

The general approach to the therapy of nephrotic edema is listed in Table 2-5. As pointed out, nephrotic patients may be particularly susceptible to diuretic agents that act in the distal nephron. Distal-acting diuretics of the potassium-sparing variety also prove to be helpful in the management of the hypokalemia that may occur. These patients also respond to mineralocorticoid antagonists.

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CHAPTER 3

Pathogenesis and Management of Metabolic Acidosis and Alkalosis

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Acid–base disorders occur commonly in clinical medicine. Although the degree of acidosis or alkalosis that results is rarely life threatening, the careful evaluation of the acid–base status of the patient often provides insight into the underlying medical problem. Moreover, the pathophysiology and differential diagnosis of these disorders can be approached quite logically with a minimum of laboratory and clinical data. An effective approach to clinical acid–base disorders is accomplished most easily with a stepwise pathophysiologic approach.

Human acid–base homeostasis normally involves the tight regulation of CO_2 tension by respiratory excretion and plasma bicarbonate [HCO_3^-] concentration by renal HCO_3^- reabsorption and elimination of protons (H^+) produced by metabolism. The pH of body fluids (which can be sampled easily) is determined by the CO_2 tension (in arterial blood, $Paco_2$) and the [HCO_3^-]. Primary derangements of CO_2 tension are referred to as respiratory disturbances, whereas primary derangements of [HCO_3^-] are called metabolic disturbances (1).

In this chapter, we first review acid–base chemistry and physiology

and then present a pathophysiologic approach to the diagnosis and management of metabolic acidosis and alkalosis.

Acid–Base Chemistry and Physiology

The chemistry of acids, bases, and buffers and the normal physiology of acid and bicarbonate excretion (2,3) are described in detail in several excellent reviews and are summarized only briefly in this section.

BUFFERING

Clinical acid–base chemistry basically is the chemistry of buffers. For clinical purposes, we may define an acid as a chemical that donates an H^+ and a base that accepts an H^+. For any acid (HA), we can define its strength or tendency to donate H^+ by its dissociation constant *K* according to the relationship:

$$[HA] = K_{eq} \times [H^+][A^-] \quad (3.1)$$

If we rearrange this equation and apply a log transformation, we arrive at the familiar relationship:

$$pH = pK + \log_{10}[A^-]/HA \quad (3.2)$$

Buffering refers to the ability of a solution containing a weak or poorly dissociated acid and its anion (a base) to resist change in pH when a strong acid (i.e., highly dissociated acid) or alkali is added. To illustrate this important point, suppose 1 mL of 0.1 mol/L HCl is added to 9 mL of distilled water, the $[H^+]$ would increase from 10^{-7} to 10^{-2} mol/L. In other words, the pH would fall from 7.0 to 2.0. However, if we added 1 mL of 0.1 mol/L HCl to 9 mL of a 1 mol/L phosphate *buffer* (p*K* = 6.9) at pH 7.0, most of the dissociated H^+ from HCl would combine with dibasic phosphate (HPO_4^{2-}) and only slightly change the ratio of dibasic to monobasic ($H_2PO_4^-$) phosphate. In fact, the pH would fall by only about 0.1 pH units. The addition of acid has been *buffered* by the phosphate dissolved in water. Another way to think about this is that the pH was stabilized by substances that bound the free H^+ released by the HCl, in this case the phosphate. Such substances are called *buffers* (4).

Biochemical Determinants of pH

The bicarbonate buffer system is the most important buffer in the extracellular space in humans. Proteins and inorganic phosphate are less important buffers. Inorganic phosphate is probably the most important buffer in the intracellular space followed by bicarbonate and intracellular proteins. Although intracellular pH (pHi) is probably more important in predicting physiologic and clinical consequences than extracellular pH (5), it is difficult to measure in vivo without using sophisticated investigational techniques, such as ^{31}P nuclear magnetic resonance (NMR) spectroscopy (6), laser scanning cytometry (7), and fluorescence lifetime imaging (8), which are not available for routine clinical applications. Therefore, our clinical efforts are focused on classifying disease states based on what is measurable, that is, extracellular pH. In particular, our attention focuses on the bicarbonate buffer system (2). We can assume that equilibrium conditions apply because there is abundant carbonic anhydrase in blood. Therefore, we can view the bicarbonate buffer system as the equilibrium reaction:

$$H^+ + HCO_3^- \overset{K_{eq}}{\rightleftharpoons} H_2CO_3 \quad \text{or} \tag{3.3}$$

$$[H^+] = K_{eq} \times [H_2CO_3]/[HCO_3^-] \tag{3.4}$$

H_2CO_3 is defined by the partial pressure of CO_2 and the solubility of CO_2 in physiologic fluids, which is a constant S to all intents and purposes. We can therefore rearrange this equation as

$$[H^+] = K \times (S \times Pco_2)/[HCO_3^-] \tag{3.5}$$

which is attributed to Henderson in 1909.

Taking the antilog of both sides gives the following:

$$pH = pK + \log_{10}[HCO_3^-]/(S \times Pco_2) \tag{3.6}$$

which is called the Henderson–Hasselbalch equation, first described by Hasselbalch in 1916. In blood at 37°C, the pK of the bicarbonate buffer system is 6.1 and the solubility coefficient for CO_2 is 0.03. Therefore, we can simplify our expression to

$$pH = 6.1 + \log_{10}[HCO_3^-]/[0.03 \times Paco_2] \tag{3.7}$$

In the above equations, [HCO_3^-] is expressed in mM (or mEq/L) and $PaCO_2$ is expressed in torr (or mm Hg). Convenient expression allows us to view acid–base disorders as being attributable to the numerator of the ratio (metabolic processes), the denominator (respiratory processes), or both (mixed or complex acid–base disorders) (Fig. 3-1) (1).

Total Body Acid–Base Metabolism

A myriad of enzymatic reactions involve the loss or gain of protons that occurs with ongoing catabolism and anabolism. However, one simply has to examine the initial substrates and final products to understand whether acid or base is produced. To do this, it is helpful to think of acids and bases as "Lewis" acids and bases, in other words, to consider acids as electron acceptors rather than proton donors. In concrete terms, acid is generated when a substrate is metabolized to something more anionic (e.g., glucose is metabolized to lactate through the Embden–Meyerhof glycolytic pathway). Conversely, if a substrate is metabolized to something more cationic (e.g., lactate is metabolized to CO_2 and H_2O via the tricarboxylic acid [TCA] cycle), then acid is consumed (9). Because of the importance of the bicarbonate buffer system in the overall acid–base homeostasis, we generally consider the addition of a proton as equivalent to the decrease in total body HCO_3^- and loss of a proton as a gain in HCO_3^- (9).

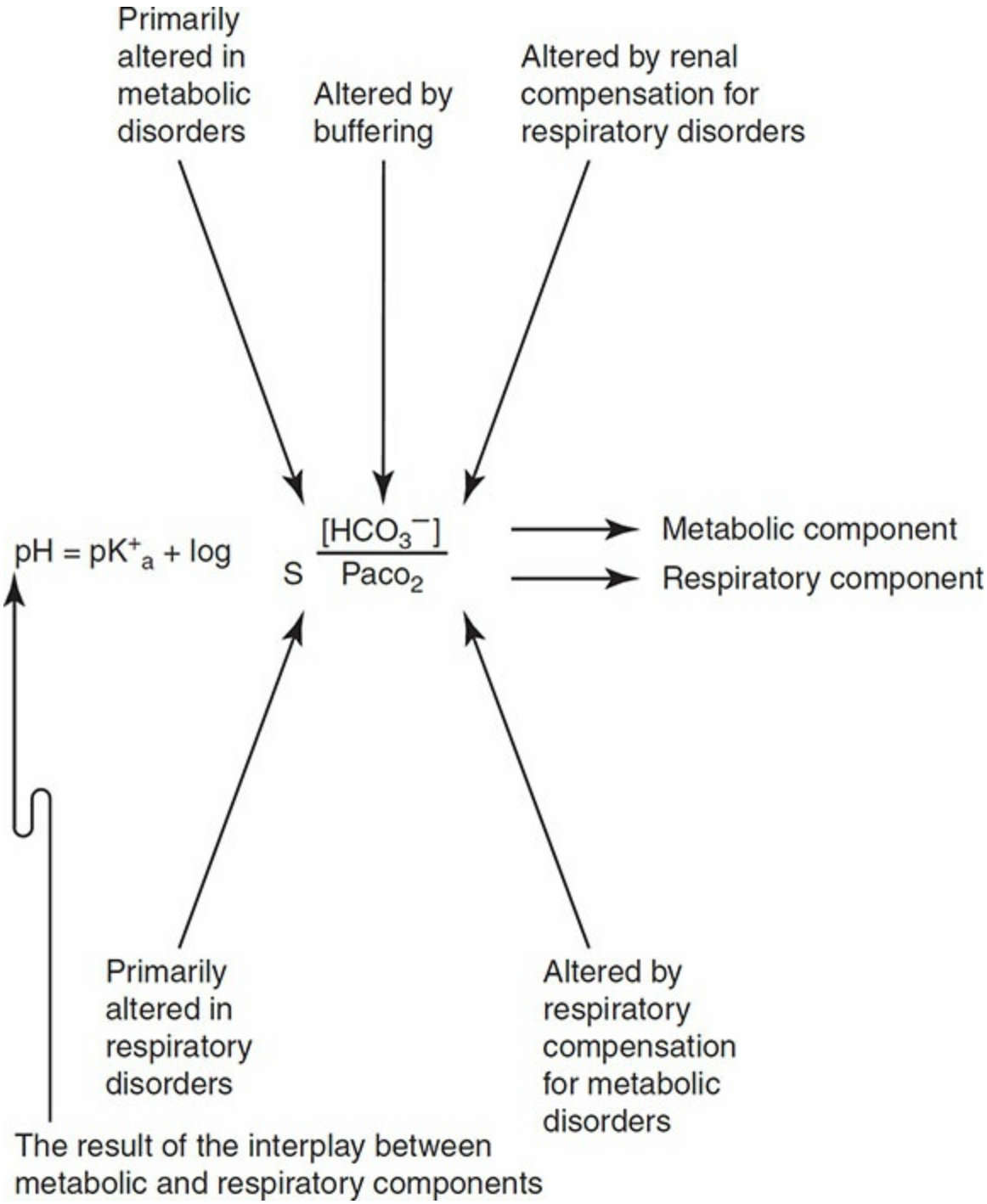

Figure 3–1 Modified Henderson–Hasselbalch equation, portraying the interacting effects of the primary acid–base disturbance and the secondary mechanisms on the pH.

This approach to understanding acid–base metabolism has led to its practical application in treating the acidosis of chronic renal failure with peritoneal dialysis. Although the lactate-based dialysate (generally at about 35 mM) has a pH of only 6.0, virtually all of the lactate is ionized; bicarbonate is generated and the acidosis is corrected via metabolism largely through the TCA cycle to CO_2 and H_2O (10).

Renal Acid Excretion

GENERAL CONSIDERATIONS

In adult men studied at sea level, the kidneys regulate the [HCO_3^-] at approximately 24 mM and the lungs control the $PaCO_2$ at about 38 torr, thus producing an arterial pH of approximately 7.42; for women, the corresponding values for [HCO_3^-], $PaCO_2$, and pH are 24 mM, 37 torr, and 7.43, respectively (11). The kidneys regulate plasma [HCO_3^-] and acid–base balance by reclaiming filtered HCO_3^- and generating new HCO_3^- to replace that lost internally in titrating metabolic acid and externally (e.g., from the gastrointestinal [GI] tract). A normal "Western diet" generates approximately 1 mmol of acid per kilogram of body weight per day. This acid load must be excreted by the kidney to maintain acid–base homeostasis. The easiest way to understand the molecular processes involved in renal acid excretion is to separate renal acid–base handling itself into two functions: bicarbonate reabsorption and net acid excretion (NAE) (12).

RENAL CELLULAR MECHANISMS OF PROTON EXTRUSION

In recent years, our understanding of the renal cellular transport proteins that effect H^+ extrusion has expanded significantly. We now know that in addition to the sodium–proton exchanger (Na^+/H^+ exchanger) that exchanges one H^+ for one sodium molecule, the sodium phosphate symporter that transports one sodium with one monobasic phosphate molecule, and the vacuolar H^+ ATPase that directly pumps H^+ into the tubular lumen (13–19), other transport proteins may be of considerable importance. These other transport proteins include the family of chloride–bicarbonate symporters and exchangers, the "colonic" H^+/K^+ ATPase, and the Na^+/K^+ ATPase (20–23). These transport proteins are expressed to different degrees in the different nephron segments depending on the function of the cell type within that nephron section.

On a more global basis, there is a tight link between acid secretion and the reclamation of filtered bicarbonate as well as the production of new bicarbonate by the kidney. For example, the reclamation of HCO_3^- filtered

from the blood occurs when HCO_3^- formed inside the renal tubular cells by either H^+ secretion or ammonium (NH_4^+) synthesis is transported back into the blood via the basolateral $Na^+(HCO_3^-)_3$ symporter (23) or a Cl^-/HCO_3^- antiporter (13,24). Alternatively, bicarbonate can be secreted by certain cells of the collecting duct in exchange for chloride (25).

RENAL ACID–BASE METABOLISM

Base Reabsorption

When plasma is filtered at the glomerulus, HCO_3^- enters the tubule lumen. Mechanistically, each HCO_3^- that is reclaimed requires the epithelial secretion of one H^+. This is accomplished largely by an Na^+/H^+ exchanger on the luminal membrane, although an electrogenic H^+ ATPase also may be involved. On an organ physiology level, HCO_3^- reabsorption can be considered in terms of the *plasma threshold* (PT) for bicarbonate, that is, the plasma HCO_3^- concentration at which HCO_3^- begins to appear in the urine. In terms of the maximal net activity of tubular HCO_3^- reabsorption (also called T_{max}), assuming glomerular filtration rate (GFR) of 100 mL/min and a plasma [HCO_3^-] of 24 mM, the renal tubules must secrete about 2.4 mmol of H^+ per minute to reclaim all of the filtered HCO_3^-. Therefore, HCO_3^- reclamation by the tubules involves a tremendous amount of H^+ secretion. Bicarbonate reclamation is coupled tightly to sodium reabsorption and also is sensitive to a number of other influences. As the T_{max} for HCO_3^- increases, the PT for HCO_3^- increases. Conversely, decreases in T_{max} result in decreases in the PT. In particular, states of extracellular fluid (ECF) expansion and decreases in P_{CO_2} decrease the apparent T_{max} for HCO_3^-, whereas ECF contraction and increases in P_{CO_2} increase the apparent T_{max} for HCO_3^-. Parathyroid hormone (PTH) inhibits proximal tubule HCO_3^- reabsorption and lowers the apparent T_{max} and PT for HCO_3^-. Most (but not all) of this HCO_3^- reabsorption (about 85–90%) occurs in the proximal tubule (3).

Carbonic anhydrase that is present both intracellularly and on the tubular surface of the brush border of the proximal tubule allows the

secreted H^+ that combines with tubular fluid HCO_3^- to form H_2CO_3. This H_2CO_3 rapidly dissociates to form H_2O and CO_2 that can readily permeate proximal tubule cell membranes. Intracellularly, carbonic anhydrase catalyzes the formation of H_2CO_3 again, which subsequently dissociates into HCO_3^- and H^+. Finally, HCO_3^- leaves the cell via several bicarbonate transport proteins, including the $Na^+(HCO_3^-)_3$ symporter and the Cl^-/HCO_3^- exchanger (26). The secreted H^+ also titrates citrate, another form of alkali (27). This process is shown schematically in Figure 3-2.

Net Acid Excretion

NAE is the net amount of H^+ eliminated from the body. If we postulate that an excreted HCO_3^- molecule negates the value of an excreted H^+, then we can consider NAE by the kidney to be the amount of H^+ (both buffered and free) excreted in the urine minus the amount of HCO_3^- excreted in the urine. As discussed, H^+ secretion into the tubule lumen mandates 1:1 stoichiometric HCO_3^- transport across the basolateral segment into the extracellular space; therefore, NAE represents the amount of new HCO_3^- generated by the kidneys and added to the body stores.

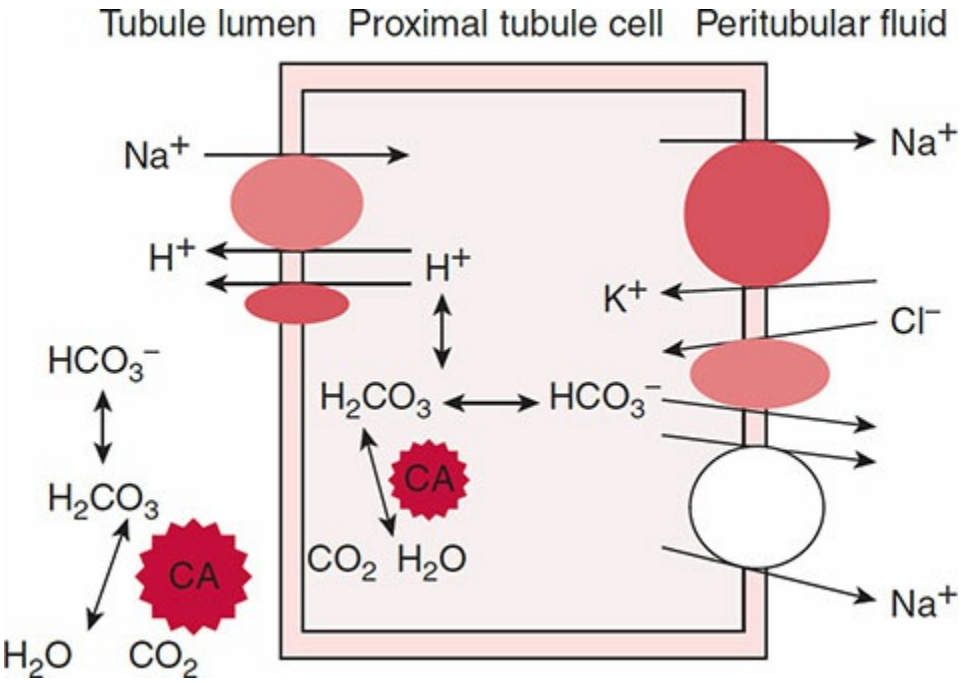

Figure 3–2 Schematic depicting proximal tubule HCO_{3-} reclamation process. Two vehicles for apical H^+ secretion: Na^+/H^+ exchanger (*lightly shaded ellipse*) and H^+

ATPase (*filled ellipse on apical side*) are shown. Some basolateral ion pumps and exchangers, including the Na^+/K^+ ATPase (*filled circle*), $Na^+(HCO_{3-})_3$ symporter (*open circle*), and HCO_{3-}/Cl^- exchanger (*lightly shaded ellipse*), are also shown. The role of carbonic anhydrase (CA) both in the tubular cell and on the brush border in HCO_{3-} reabsorption also is depicted.

NAE is accomplished primarily through elimination of titratable acid (which is mostly phosphate) and nontitratable acid (in the form of NH_4^+) (12). These terms refer to clinical chemistry titration techniques by which known amounts of alkali were added to urine until a color change with a pH indicator (e.g., phenolphthalein) occurred. This color change occurred above the p*K* for the phosphate buffer system but below the p*K* for ammonia–ammonium (about 9). It must be stressed that the NAE is relatively insensitive to the urine pH. This concept is illustrated by the observation that addition of 1 mmol of HCl to 1 L of distilled water results in a pH of 3 (corresponding to an H^+ concentration of 10^{-3} mol/L). Therefore, in the extreme case (i.e., no buffers in the urine at all), extremely acidic urine (i.e., a very low pH) could eliminate very few protons from the body. There are several clinical settings discussed later in which acidic urine is elaborated, but NAE is insufficient. NAE requires the adequate function of both the proximal tubule to synthesize NH_4^+ (which generates an HCO_3^- molecule) and the distal tubule and collecting tubules where H^+ and NH_4^+ secretion occur (26).

Proton secretion by the distal nephron involves the production of an electrogenic gradient that favors H^+ secretion produced by removal of sodium from the luminal fluid and direct pumping of H^+ into the tubular lumen. The latter is accomplished by the activities of vacuolar H^+ ATPase and the H^+/K^+ ATPase in type A intercalated cells. Notably, chloride exchange with bicarbonate on the basolateral side of these distal tubular cells allows for proton secretion to be translated into bicarbonate addition to the blood. Finally, the epithelial membrane must not allow back leak of H^+ or loss of the electrogenic gradient. Under normal circumstances, humans can elaborate a urine pH as low as 4.4, representing a 1,000:1 gradient of H^+ between tubular fluid and cells. However, the excretion of NH_4^+, which is discussed later, is of much greater importance in terms of NAE than is the level of urine pH achieved (28).

NAE is sensitive to a variety of factors, including the plasma K concentration (increases in plasma K decrease NH_4^+ excretion, whereas

decreases enhance H^+ secretion by the distal nephron) and the effects of aldosterone. By stimulating the renin–angiotensin–aldosterone system, ECF contraction enhances distal acid secretion (29).

Ammonium Metabolism

The traditional view that NH_4^+ excretion was determined by simple passive trapping of NH_4^+ in the tubular lumen has been revised considerably. Recent studies have led to new insights into mechanisms of renal ammonia transport and metabolism (30). Although many of these proteins are primarily involved in the transport of H^+ or K^+, they also transport NH_4^+ (31). The role of aquaporins and the identification of mammalian Rh glycoproteins (discussed below) are two new exciting areas of research in understanding ammonia transport in normal acid–base homeostasis (30). Importantly, proximal tubule cells deaminate glutamine to form alpha ketoglutarate ($^{\alpha}KG$) and NH_4^+. Proximal tubule cells then secrete NH_4^+ into the lumen, probably via substitution for an H^+ using the luminal Na^+/H^+ antiporter. A key feature is that the further metabolism of $^{\alpha}KG$ generates a new HCO_3^- molecule. Therefore, complete metabolism of each glutamine produces two NH_4^+ and two HCO_3^- ions. Ammonium is later reabsorbed in the thick ascending limb of Henle (via substitution for K^+ using the $Na^+/K^+/2Cl^-$ cotransporter in the apical membrane), then across the basolateral membrane via the sodium–hydrogen exchanger isoform 4 (NHE4) to the tubular interstitial space (32). This ultimately results in the increase in the medullary concentration of NH_4, which is highest in the inner medulla. This NH_4^+ is then taken up by distal convoluted tubule and collecting duct cells, substituting for K^+ using the basolateral Na^+/K^+ ATPase of the inner medullary collecting duct or as NH_3 (after losing H^+) utilizing ammonia-specific transporters Rhesus Glycoproteins (Rhbg and Rhcg) along with a component of diffusive absorption. Collecting duct ammonia secretion involves parallel secretion of NH_3 utilizing Rhcg of the apical membrane or by its diffusive capacity along with cytosolic H^+ generated by carbonic anhydrase II–mediated mechanism. H^+ secretion across the apical membrane is primarily

mediated by H^+-ATPase and H^+-K^+ ATPase pumps. In the tubular lumen, H^+ titrates luminal NH_3 to form NH_4^+ and maintains low NH_3 concentration in the urinary space, which is necessary for continued NH_3 secretion (33). The net generation of any HCO_3^- from $^{\alpha}$KG metabolism is ultimately dependent on the excretion of NH_4^+. This is because if this NH_4^+ molecule is not excreted in the urine but rather is returned via the systemic circulation to the liver, it is used to form urea at the expense of generating an H^+. Thus, the HCO_3^- molecule that was generated by the metabolism of the $^{\alpha}$KG will be neutralized, and no change in acid–base status will occur (30).

Difficulties in the routine clinical measurement of urinary NH_4^+ concentrations have delayed our appreciation of its importance in net acid–base balance during pathophysiologic conditions including chronic metabolic acidosis or acid loads (31). However, recent observations by Batlle et al. (34) suggest that the urinary [NH_4^+] may be inferred fairly accurately by calculations based on urinary electrolyte concentrations as is discussed subsequently.

Although the topic under discussion is “renal” acid–base metabolism, the liver may be significantly involved. Hepatic glutamine synthetase expression appears to be regulated by pH as well as protein ingestion (35–37). Perhaps more importantly, administration of amino acids via a parenteral rather than the enteral route often is accompanied by acid retention (38).

Clinical Approach to Acid–Base Disorders

Metabolic Acidosis

DEFINITIONS AND CAUSES

Metabolic acidosis is a systemic disorder characterized by a primary decrease in [HCO_3^-]. This may occur in three ways: (a) the addition of strong acid that is buffered by (i.e., consumes) HCO_3^-; (b) the loss of HCO_3^- from body fluids, usually through the GI tract or kidneys; and (c) the rapid addition to the ECF of nonbicarbonate solutions (dilutional

acidosis). No organic anion is generated when HCO_3^- is lost or diluted. Reciprocal increases in the serum chloride concentration occur to preserve electroneutrality. Thus, these forms of metabolic acidosis generally are referred to as hyperchloremic or nonanion gap metabolic acidosis. When an organic acid consumes HCO_3^-, its organic anion is generated and may be retained in the ECF and serum. The serum chloride concentration does not increase with organic acidosis. An increase in the anion gap marks the existence and concentration of the organic anion (39).

DEFENSE OF SYSTEMIC pH DURING METABOLIC ACIDOSIS

Buffering

The hallmark of metabolic acidosis is a fall in plasma [HCO_3^-]. We stress that the fall in [HCO_3^-] always is mitigated by the participation of other buffers in both the ECF and the intracellular fluid (ICF). Roughly one-half of an administered acid load is buffered by nonbicarbonate buffers (40). Bone is an important buffer pool in states of chronic metabolic acidosis. In fact, the leaching of calcium from bone is one of the major deleterious effects of chronic metabolic acidosis (41–43).

Respiratory Compensation

A fall in $PaCO_2$ is a normal compensatory response with simple metabolic acidosis. The failure of this normal adaptive response is indicative of the presence of respiratory acidosis in the setting of a complex or mixed acid–base disturbance. Conversely, an exaggerated fall in $PaCO_2$ producing a normal pH indicates the presence of respiratory alkalosis in the setting of a complex or mixed acid–base disturbance (44). The mechanism by which metabolic acidosis induces hypocapnia appears to be mediated in part by peripheral pH receptors in the carotid body, but mostly by central nervous system (CNS) pH receptors. This point is supported by the time course, specifically the temporal delay observed for respiratory compensation seen in experimental metabolic acidosis (45). The degree of chronic compensation varies from person to person; however, based on a large volume of clinical data, we can state with some confidence that the appropriate fall in $PaCO_2$ (in torr) should be 1 to 1.5 × the fall in [HCO_3^-]

(in mM) (44). Oral acid loading in normal subjects produced a rapid fall in P_{CO_2} that reached a steady state after 30 minutes that was 0.85 times the fall in HCO_3^-, thus providing direct evidence for the rapid respiratory response to metabolic acidosis in humans (46).

Correction

The kidney provides the mechanism for the third line of defense to pH changes. However, this mechanism is rather slow compared to buffering (which begins immediately) and respiratory compensation (which begins within 15–30 minutes), since it takes up to 5 days to become maximal. NAE is increased in response to either metabolic acidosis (unless the kidney is the cause) or respiratory acidosis. This increase in NAE is largely NH_4^+ excretion because titratable acid excretion is limited by the amount of excreted phosphate, which changes very little. Metabolic acidosis increases the processing of glutamine into NH_4^+, which, in turn, leads to enhanced generation of HCO_3^-, by both transcriptional and translational regulations of key enzymes in this pathway (47). Chronic metabolic acidosis increases renal endothelin-1 activity that activates the NHE3 sodium–hydrogen ion antiporter on the proximal tubule brush border (48). Thus, both the generation of new HCO_3^- via the glutamine system and the enhancement of HCO_3^- reabsorption and titratable acid formation are stimulated. Of interest, the hypocapnia that occurs because of respiratory compensation actually limits renal correction in metabolic acidosis (49). Note that renal correction never corrects the pH to normal until the disorder causing HCO_3^- loss or acid generation is halted.

BIOCHEMICAL AND PHYSIOLOGIC EFFECTS OF METABOLIC ACIDOSIS

Mild degrees of acidemia generally are well tolerated, at least acutely, and even may afford some physiologic advantages, such as favorable oxygen delivery from hemoglobin. However, with marked acidemia, pH < 7.10, myocardial contractility is depressed and peripheral resistance falls (50,51). These manifestations may be a result of the effect of acidosis to depress both vascular and myocardial responsiveness to catecholamines as well as innate myocardial contractility. Both myocardial β-adrenergic

receptor density and physiologic responses to β agonists appear to be decreased by metabolic acidosis (52,53).

Clearly, metabolic acidosis induces an intracellular acidosis in myocytes (48,49). This intracellular acidosis in turn impairs contractile responses to normal or even elevated cytosolic calcium concentrations (54,55). Alterations in troponin I–troponin C interactions mediated by low pH appear to shift the sensitivity of troponin C to calcium (56). Additionally, impairment of actin–myosin cross-bridge cycling caused by increases in the concentration of inorganic phosphate in the monovalent form may be involved in the decreased calcium sensitivity and contractile dysfunction seen with acidosis (57). The increase in monovalent inorganic phosphate results both from the acidic environment, which increases the ratio of monovalent to divalent inorganic phosphate, and from an impairment of myocardial energy production, which increases the total intracellular concentration of inorganic phosphate (58,59). Metabolic acidosis and hypoxia appear to additively or synergistically impair myocardial function, a phenomenon consistent with the monovalent inorganic phosphate hypothesis (60). The vasodepressor effect of acidosis likely results from similar molecular mechanisms (61).

CLINICAL FEATURES

Clinically, one can observe an increase in ventilatory effort with even mild degrees of acidosis. With severe metabolic acidosis (e.g., pH < 7.20), respirations become extremely deep and rapid (Kussmaul). Mild degrees of acidosis do not appear to markedly impair hemodynamic stability, at least in subjects with otherwise normal cardiovascular function. However, severe metabolic acidosis may lead to hypotension, pulmonary edema, and, ultimately, ventricular standstill (50,62,63). Chronic metabolic acidosis, even if fairly mild, causes hypercalciuria and bone disease as bone buffering of acid leads to marked calcium losses from the bone because of increases in prostaglandin E2 (PGE2) production (64). This aspect is extremely important in determining treatment of renal tubular acidosis (RTA) or the acidosis of chronic renal failure.

LABORATORY FINDINGS

Simple metabolic acidosis is characterized by a decrease in blood pH, $[HCO_3^-]$, and P_{CO_2} (through compensation). Note that a failure to lower

the P_{CO_2} by 1 to 1.5 × the fall in $[HCO_3^-]$ indicates the coexistence of respiratory acidosis (44). The clinical implications of this are quite profound because this failure of compensation may signify impending severe respiratory failure. Serum electrolytes reveal a fall in $[TCO_2]$. Acidosis tends to shift potassium out of cells in a rather complex manner (65), and renal potassium excretion tends to increase in many states of metabolic acidosis. Normal or increased serum potassium in the face of decreased total body potassium stores occurs commonly in cases of metabolic acidosis (Chapter 5) (65). Some states of metabolic acidosis are characterized by the retention of an organic anion generated in concert with HCO_3^- consumption (organic acidosis), whereas others are not (hyperchloremic). The screening of plasma for such organic anions is not practical on a routine, immediate basis; thus, a calculation performed on the serum electrolytes called the serum anion gap (SAG) is employed (66).

Serum Anion Gap

The SAG is a concept used in acid–base pathophysiology to infer whether an organic or mineral acidosis is present. We use the routine venous blood serum electrolytes to calculate the SAG (58):

$$SAG = [Na^+] - [Cl^-] - [TCO_2] \tag{3.8}$$

Here we use TCO_2 as an index of HCO_3^-. We define unmeasured cations (UCs) as cations that are not Na^+ (e.g., K^+, Mg^{2+}, Ca^{2+}) and unmeasured anions (UAs) as anions that are not Cl^- or HCO_3^- (e.g., SO_4^{2-}, $H_2PO_4^-$, HPO_4^{2-}, albumin, organic anions). Thus, electroneutrality demands that

$$[Na^+] + [UC] = [Cl^-] + [TCO_2] + [UA] \tag{3.9}$$

where the UA and UC concentrations are expressed in mEq/L rather than mmol/L. Putting formulas (3.8) and (3.9) together, we see that

$$SAG = [UA] - [UC] \tag{3.10}$$

Normally, the SAG is about 9 (6–12 mEq/L). Although the SAG is used routinely in the differential diagnosis of metabolic acidosis, we stress that it is a relative rather than absolute indicator of the underlying pathophysiology. Note that the maintenance of stoichiometry (i.e., 1 mEq

increase in anion gap for every 1 mmol decrease in HCO_3^-) depends on the clearance mechanisms for the anion as well as the myriad factors that influence HCO_3^- concentration. Therefore, some organic acidoses may manifest trivial or even no increase in the anion gap, whereas some hyperchloremic acidoses may have coincidental increases in the anion gap. This must be kept in mind when evaluating the differential diagnosis of metabolic acidosis. However, a major increase in the anion gap (e.g., SAG >26 mEq/L) always implies the existence of an organic acidosis (67).

If we look at the changes in the SAG and compare it to changes in HCO_3^- concentrations, additional insights can be made. This is often referred to as the "Delta gap" = Delta SAG – Delta Serum [HCO_3^-] or the "Delta–Delta." It takes advantage of the assumption that with a pure organic metabolic acidosis, the fall in HCO_3^- and the increase in SAG should be equal (with the disclaimers stated above). Ergo, if the Delta gap is very positive (i.e., >6), it suggests that there must be another process generating alkali. Conversely, if the Delta gap is very negative (i.e., <−6), it suggests that there is another source of acid that does not result in an increase in SAG, ergo a concomitant nonanion gap metabolic alkalosis.

Urine Anion Gap

To address a different problem, urinary electrolytes have been used to estimate the quantity of NH_4^+ in the urine, a measurement that has been difficult to develop into a routine clinical test. The concept is quite similar to that described for the SAG. In the urine, because of electroneutrality

$$[Na^+] + [K^+] + [UC] = [Cl^-] + [UA] \quad (3.11)$$

When urine pH is <6, UA does not include appreciable amounts of HCO_3^-, but consists primarily of phosphate ($H_2PO_4^-$ more than HPO_4^{2-}) and, to a lesser degree, sulfate (SO_4^{2-}) and organic anions. UC is made up mostly of NH_4^+. Therefore, if we define the urinary anion gap (UAG) as

$$UAG = [Na^+] + [K^+] - [Cl^-] \quad (3.12)$$

then we see that this is determined largely by the amount of NH_4^+ in the urine that holds true in clinical studies of metabolic acidosis (34). In contrast to the SAG, which is useful in many settings of clinical acid–base

diagnosis and therapy, the UAG has a very narrow clinical application in the differentiation of renal from nonrenal causes of nonanion gap metabolic acidosis (68).

DIFFERENTIAL DIAGNOSIS OF METABOLIC ACIDOSIS

The differential diagnosis of metabolic acidosis generally is approached clinically by using the SAG. Those acidosis states associated with retention of an organic anion are classified as increased anion gap or simply anion gap metabolic acidosis. Those acidosis states not associated with retention of an organic anion are classified as nonanion gap or hyperchloremic metabolic acidosis (66). These disorders are listed in Table 3-1.

CAUSES OF HYPERCHLOREMIC METABOLIC ACIDOSIS

Gastrointestinal Loss of HCO_3^-

Diarrhea

Diarrhea is the most common cause of hyperchloremic metabolic acidosis and always should be considered early in the differential diagnosis. The concentration of HCO_3^- in diarrheal fluid is generally greater than that of plasma. In the extreme case of cholera, patients may lose up to 20 L/day of fluid containing 30 to 50 mEq/L of HCO_3^- (69). However, hypovolemic shock likely will cause lactic acidosis and increase the anion gap in that situation. Ileostomy fluid is also rich in HCO_3^-, especially early after construction (70).

Table 3–1 Differential Diagnosis of Metabolic Acidosis

Normal Anion Gap (Hyperchloremic)	Increased Anion Gap (Organic)
Gastrointestinal Loss of HCO_3^-	**Increased Acid Production**
Diarrhea	Lactic acidosis
Intestinal fistula or drainage	Diabetic ketoacidosis
Anion exchange resins	Starvation
	Alcoholic

	ketoacidosis
Renal Loss of HCO_3^- Renal tubular acidosis Carbonic anhydrase inhibitors Hypoaldosteronism K-sparing diuretics	Inborn errors of metabolism Toxic alcohol ingestion Salicylate intoxication Other intoxications Failure of acid excretion
Miscellaneous Recovery from ketoacidosis Dilutional acidosis Addition of HCl Parenteral alimentation Sulfur ingestion	Acute renal failure Chronic renal failure — — — —

The diagnosis of diarrheal loss of HCO_3^-, however, may be difficult in the very young or very old (71). In the former case, the distinction between diarrhea and an underlying RTA is extremely important. The UAG may be very helpful in this setting. Patients with diarrhea as a cause of metabolic acidosis typically have a very negative UAG (i.e., urinary chloride exceeds the sum of urinary $NA^+ + K^+$ by >10 mEq/L) reflecting the presence of ample urinary NH_4^+ concentrations, whereas patients with all forms of distal RTA have positive UAGs reflecting the inadequate urinary NH_4^+ concentrations present (34).

Gastrointestinal Drainage and Fistulas

The succus entericus and pancreatic and biliary secretions are rich in HCO_3^- and poor in Cl^-. The succus entericus has a daily volume of 600 to 700 mL but may be increased in disease states. Biliary secretions amount to >1 L/day of fluid, with [HCO_3^-] approaching 60 mmol/L. Pancreatic secretion may exceed 2 L/day, with [HCO_3^-] approaching 120 mmol/L. Therefore, it is not surprising that GI drainage or fistulas could cause significant metabolic acidosis (72).

The technique of simultaneous kidney/pancreas transplant (SPK) has been used for the treatment of type I diabetic patients with end-stage renal

been used for the treatment of type I diabetic patients with end-stage renal disease (73). When urinary drainage of the HCO_3^--rich exocrine secretions of the pancreatic allograft is employed rather than enteric drainage, this addition of pancreatic exocrine fluid causes urinary loss because the bladder cannot absorb the pancreatic secretion. Thus, SPK with urinary drainage is often associated with significant normal anion gap metabolic acidosis (74,75). Many of these patients require sodium bicarbonate supplementation (as high as 100–150 mEq/day) on a chronic basis. The incidence of metabolic acidosis can be significantly lowered with enteric drainage compared to urinary drainage because the intestine can absorb the HCO_3^- (76).

Urinary Diversion to Bowel

Patients may require urinary diversion from normal egress through the bladder for a variety of reasons. Approaches to this include the creation of an ileal loop conduit or, less commonly, drainage of the ureters into the sigmoid colon (ureterosigmoidostomy) (77). Although metabolic acidosis can develop with both procedures, it is more severe with ureterosigmoidostomy. The pathophysiology for both situations is the bowel mucosal secretion of HCO_3^- in exchange for Cl^- during water reabsorption, which may lead to significant HCO_3^- losses in the GI tract effluent (72). Newer reconstructive procedures including ileal neobladders, which minimize the time of contact between urine and bowel mucosa, have been successful in limiting HCO_3^- loss (78).

Chloride-containing Anion Exchange Resins

Cholestyramine is a nonabsorbable anion exchange resin used to bind bile acids in the gut for a variety of purposes, including the treatment of obstructive liver disease as well as hypercholesterolemia and the management of acute diarrhea in children (79). However, this resin has some affinity for HCO_3^- and may exchange Cl^- for HCO_3^- across the bowel mucosa. In conditions of renal insufficiency where new HCO_3^- generation is impaired, in volume depletion or patients taking spironolactone, hyperchloremic metabolic acidosis has been reported (80).

Calcium or Magnesium Ingestion

The divalent cations, calcium or magnesium, are absorbed incompletely in the GI tract. If large amounts of these cations are ingested in soluble form (e.g., as the Cl_2 salts), then the unabsorbed Ca^{2+} or Mg^{2+} reacts with HCO_3^-, which has been exchanged across the mucosa for Cl^-, to form an insoluble salt. Thus, plasma HCO_3^- falls to a moderate degree (81).

Renal Loss of HCO_3^-

Renal Tubular Acidosis

RTA refers to a group of functional disorders characterized by impairment of renal HCO_3^- reabsorption and H^+ excretion that is out of proportion to any reduction in GFR. In many cases, the RTAs exist in the presence of a completely normal GFR. Unfortunately, a nomenclature has evolved that confuses many experienced clinicians as well as trainees and students. We provide a pathophysiologic classification of these disorders while referring to this nomenclature. RTAs can be divided into those characterized by disturbed distal nephron function (i.e., impaired NAE) and those caused by impaired proximal HCO_3^- reabsorption (82). Distal RTAs are divided into those associated with hypokalemia (83) and those associated with hyperkalemia, which may be further subdivided into RTA caused by hypoaldosteronism and RTA characterized by a general distal tubular defect (84).

Proximal Renal Tubular Acidosis

Proximal RTA, also called type II RTA, is an uncommon but very interesting disorder (85,86). Basically, the acid–base disturbance is caused by impairment in proximal tubular reabsorption of HCO_3^-, the nephron site where 85% of HCO_3^- usually is reabsorbed. The delivery of HCO_3^--rich fluid to distal nephron sites leads to substantial bicarbonaturia, when plasma levels of HCO_3^- are normal, as well as urinary losses of potassium and sodium. Thus, patients present with hypokalemia and hyperchloremic metabolic acidosis. When the plasma concentration of HCO_3^- is maintained at normal by administration of HCO_3^-, fractional HCO_3^- excretion (i.e., the fraction of filtered HCO_3^- that is excreted in the urine) exceeds 15%.

In physiologic terms, the apparent T_{max} and PT for HCO_3^- are significantly reduced in patients with proximal RTA. However, once a level of plasma HCO_3^- is achieved that is below the patient's PT for HCO_3^-, renal acid handling is normal. In other words, NAE equals dietary and endogenous acid production rates and the subject comes into a steady state of acid–base balance, albeit at a moderately reduced plasma HCO_3^- concentration (and a mild reduction in systemic pH). Because a steady state in acid handling is achieved, patients with proximal RTA have less severe acidosis as well as less nephrocalcinosis (which results from bone calcium mobilization from acidosis) than patients with distal RTAs (see the following).

The problem with proximal HCO_3^- reabsorption may occur independently but more commonly coexists with other defects in proximal nephron function, such as decreased reabsorption of glucose, amino acids, phosphate, and uric acid. The term Fanconi syndrome is employed when general proximal nephron function is disturbed (87). Patients with full-blown Fanconi syndrome may have severe osteomalacia and malnutrition in addition to the mild metabolic acidosis associated with proximal RTA (88). Proximal RTA may occur as a primary disorder and present in infancy or may be acquired in the course of other diseases or as a result of exposure to substances toxic to this nephron segment. A list of causes of proximal RTA is shown in Table 3-2. Treatment of this condition is approached by addressing the underlying cause, but if this is ineffective, administration of large amounts of HCO_3^- (10–15 mmol/kg/day) and potassium to compensate for ongoing potassium losses in the urine caused by the bicarbonaturia is necessary. This is necessary to avoid growth retardation in children and osteopenia, which may be produced by even mild degrees of acidemia (89).

Table 3–2 Causes of Renal Tubular Acidosis

Proximal	Distal (Hypokalemic)	Distal (Hyperkalemic)
Primary	Primary	Hypoaldosteronism
Cystinosis	Hypercalcemia	Obstructive nephropathy

		Sickle cell disease/trait
Lead toxicity	Multiple myeloma	Lupus erythematosus
Cadmium toxicity	Lupus erythematosus	Analgesic nephropathy
Mercury toxicity	Amphotericin B	Renal transplant rejection
Amyloidosis	Toluene	Cyclosporine toxicity
Multiple myeloma	Renal transplant rejection	Other interstitial disease
Nephrotic syndrome	Medullary sponge kidney	—
Medullary cystic disease	—	—
Outdated tetracycline	—	—
Injury from kidney preservation	—	—

Distal Renal Tubular Acidosis

The distal RTAs are characterized primarily by impaired NAE, which is due, at least in part, to impaired NH_4^+ excretion. The central role of impaired NH_4^+ excretion in this disorder is highlighted by a recent clinical study in which all patients with either hypokalemic distal RTA (also called type I or classic distal RTA) or hyperkalemic distal RTA (previously referred to as type IV RTA), caused by either hypoaldosteronism or a generalized tubular defect, had a positive UAG reflecting decreased NH_4^+ excretion. How NH_4^+ excretion is impaired in this diverse set of clinical disorders is still incompletely understood (84,90).

Hypokalemic distal RTA has long been considered a disorder of the collecting duct in which the quantity of H^+ secretion is inadequate to effect the necessary NAE for the subject to maintain acid–base balance. Clinically, patients with hypokalemic distal RTA present with hyperchloremic metabolic acidosis but are unable to acidify their urine (pH < 5.5 is commonly used) in response to an acid challenge. It must be stressed that the failure to acidify the urine does not fully explain the

stressed that the failure to acidify the urine does not fully explain the defect in NAE, which is primarily caused by an associated defect in NH_4^+ excretion (91). However, the failure to acidify the urine under conditions of systemic acidosis historically has been considered the clinical hallmark of hypokalemic distal RTA. The physiologic mechanisms for this impaired acidification have been a topic of interest for some time and are summarized with clinical examples in Table 3-3. Basically, four mechanisms have been suggested for impaired acidification by the distal nephron: (a) back leak through a leaky epithelium; (b) pump failure, where the H^+ ATPase cannot pump sufficient amounts of H^+; (c) voltage defect, where a favorable transepithelial voltage cannot be generated (e.g., decreased sodium delivery to the distal nephron or decreased sodium reabsorption in the distal nephron); or (d) rate defect/NH_4^+ defect, where urinary pH is reduced but NH_4^+ excretion and NAE cannot be increased to normal amounts. Hypokalemic distal RTA appears to be caused by either back leak or pump failure. Patients may have an isolated defect in the H^+/K^+ ATPase or the vacuolar ATPase (92). Hyperkalemic distal RTAs are probably caused by either voltage defect or rate defect/NH_4^+ defect (84).

A number of physiologic maneuvers have been used to examine these mechanisms clinically. The first and simplest test is that of a metered pH (i.e., using a pH meter rather than a dipstick) performed on urine collected under oil. If the subject is already acidemic (e.g., arterial pH < 7.35), then there is no need for ammonium chloride loading. In some cases, patients are able to maintain a normal plasma HCO_3^- concentration and systemic pH under most circumstances, but do not respond normally to increases in acid generation by increasing NAE. Recent studies have identified mutations in the vacuolar ATPase B1 subunit most likely responsible for this defect (93). This is called an incomplete distal RTA. If an incomplete distal RTA is suspected, ammonium chloride is administered to induce a mild case of metabolic acidosis. This test basically screens for back leak, pump failure, or voltage defects. An alternate method, the furosemide–fludrocortisone test, is a better-tolerated test for urine acidification (94). Furosemide increases distal Na^+ delivery, and the mineralocorticoid fludrocortisone increases distal H^+ secretion. Normal subjects will acidify urine to pH < 5.3 by 3 or 4 hours, whereas patients with distal RTA fail to do so. Infusion of sodium sulfate or sodium phosphate increases distal sodium delivery. The failure to lower urine pH after these maneuvers

sodium reabsorption. Another maneuver is to determine the urine to blood P_{CO_2} gradient when the patient has bicarbonaturia (urine [HCO_3^-] >100 mM) induced by HCO_3^- administration. Under conditions where bicarbonaturia is induced, H^+ secreted into the collecting duct lumen will combine with HCO_3^- and form H_2CO_3. Because carbonic anhydrase is absent in the lumen of this segment (as well as the bladder), conversion to CO_2 and water is slow and occurs largely in the urinary collecting system (i.e., renal pelvis, ureters, and urinary bladder), where the surface area for CO_2 absorption is small. This CO_2 essentially is trapped, and, when normalized for the blood P_{CO_2} (i.e., the difference between urine and blood), is a marker for the rate of distal H^+ secretion. Patients with back leak or pump failure generally have a small difference between urine and blood P_{CO_2} (<20 torr).

Table 3–3 Examples of Pathophysiologic Mechanisms in Clinical Distal Renal Tubular Acidosis

Physiologic Defect	Example
Back leak	Amphotericin B
Pump failure	Primary
Voltage defect	Amiloride
Rate defect/NH_4^+ defect	Hypoaldosteronism

Hypokalemic distal RTA may be primary or associated with other diseases, most commonly, Sjögren syndrome (95,96) and toxin exposures. These toxins may vary from wasp sting (97) to toluene inhalation, the latter capable of causing several forms of acidosis (98). A list of causes of hypokalemic distal RTA appears in Table 3-2. Some of the causes also may result in a hyperkalemic distal RTA because of a generalized tubular defect (84). Urinary obstruction and some of the autoimmune disorders are such examples (99). Perhaps the best understood cause of hypokalemic distal RTA is that due to amphotericin toxicity, which results (at least experimentally) in acidification failure owing to back leak of normally secreted H^+. Hypokalemic distal RTA usually occurs in young children in its primary form. The children never achieve a steady state of acid–base

its primary form. The children never achieve a steady state of acid–base balance; therefore, they typically present with extremely severe metabolic acidosis, growth retardation, nephrocalcinosis, and nephrolithiasis (100). Hypokalemia, which usually is present, is actually caused by the associated sodium depletion and stimulation of the renin–angiotensin–-aldosterone axis. Therefore, renal potassium losses actually decrease considerably when appropriate therapy with sodium bicarbonate is instituted. This is quite different from patients with proximal RTA, where urinary potassium losses increase considerably during therapy because of the bicarbonaturia-associated urinary K losses. Another contrasting point between proximal RTA and hypokalemic distal RTA is the amount of alkali therapy needed. Once the acute acidosis is corrected, patients with hypokalemic distal RTA only need enough alkali to account for the amount of acid generated from diet and metabolism; therefore, 1 to 3 mmol/kg/day generally is sufficient.

Hyperkalemic distal RTA from hypoaldosteronism occurs in several settings summarized in Table 3-2. Best understood is the case of either selective aldosterone deficiency or complete adrenal insufficiency. Probably the most common form of RTA is the hyporeninemic hypoaldosteronism often seen in patients with diabetic nephropathy. In patients with this form of RTA, urinary acidification as assessed by urine pH appears normal, but the patients are unable to raise NAE to appropriate levels. The defect, at least in some of these individuals, can be traced to impaired NH_4^+ synthesis in the proximal nephron, resulting directly from the hyperkalemia. Treatment of the hyperkalemia in some individuals with this disorder is sufficient to correct the disturbance in NAE. In patients with pure primary aldosterone deficiency, replacement of physiologic amounts of mineralocorticoid results in correction of the disturbance in acid–base metabolism and is both logical and appropriate therapy. However, in patients with the hyporeninemic hypoaldosteronism form, the renal defect requires pharmacologic amounts of mineralocorticoid (i.e., 5–10 times the usual physiologic dose) for efficacy. Moreover, the use of mineralocorticoid in this setting may be contraindicated because these patients often have mild renal insufficiency and tend to be total body sodium expanded rather than depleted (as is the case in the pure hypoaldosteronism form). Treatment of the hyperkalemia by increasing renal K excretion (e.g., with loop diuretics) or K excretion through the GI tract with potassium-binding resins (Kayexalate) may be the preferred approach in patients with the hyporeninemic hypoaldosteronism form.

Hyperkalemic distal RTA from a generalized tubular defect is

list of causes appears in Table 3-2. Urinary obstruction may be the most common and important cause of this form of distal RTA. Other important causes in selected populations include cyclosporine nephrotoxicity and allograft rejection in the renal transplant patient, sickle cell nephropathy in patients homozygous and occasionally heterozygous for the sickle cell gene, and many autoimmune disorders such as lupus nephritis and Sjögren syndrome. Urinary acidification is impaired similarly to the hypokalemic distal RTA patients. Also in contrast to the hypoaldosteronism form, hyperkalemia plays a less significant role in the genesis of impaired NH_4^+ excretion, which is tied directly to the impaired distal nephron function.

Carbonic Anhydrase Inhibitors

Carbonic anhydrase inhibitors such as acetazolamide inhibit both proximal tubular luminal brush border and cellular carbonic anhydrase. The net effect is a pattern of impaired HCO_3^- reabsorption similar to that of proximal RTA. These drugs are commonly used topically to treat glaucoma, but their use may be complicated by systemic effects such as hyperchloremic metabolic acidosis (101). Topiramate is an antiseizure medication widely used in children that causes a mild to moderate proximal RTA by inhibiting carbonic anhydrase (102). The use of this drug has been associated with nephrolithiasis in both adults and children because of the resultant hypocitraturia, hypercalciuria, and elevated urine pH (103).

Hypoaldosteronism

Hypoaldosteronism is associated with a hyperkalemic distal RTA. This may be produced by pharmacologic antagonism of aldosterone action or impaired aldosterone secretion. Impaired aldosterone secretion may be caused by hyporeninemia (e.g., the hyporeninemic hypoaldosteronism associated with diabetes mellitus) or may be part of adrenal insufficiency (e.g., Addison disease). With hyporeninemic hypoaldosteronism, some have suggested that the disorder is, at least in part, an adrenal disorder because plasma potassium concentrations that typically induce aldosterone secretion do not in this disorder. However, permissive amounts of angiotensin II are necessary to allow potassium to be an effective aldosterone secretagogue (104,105).

K-Sparing Diuretics

Potassium-sparing diuretics, which either block aldosterone action (e.g., spironolactone, eplerenone) or impair distal nephron sodium reabsorption (e.g., amiloride, triamterene), also may produce a hyperchloremic acidosis in concert with hyperkalemia (106,107). The observation that aldosterone antagonists may ameliorate the progression of congestive heart failure (108) has led to more widespread use, but careful monitoring of plasma potassium is necessary (109).

Miscellaneous Causes of Hyperchloremic Acidosis

Recovery from Ketoacidosis

Although diabetic ketoacidosis (DKA) is one of the best described forms of increased anion gap metabolic acidosis, many patients during recovery from DKA may eliminate the organic anions through renal clearance faster than their acidosis corrects, leaving them with a nonanion gap or hyperchloremic metabolic acidosis (110). This also may occur in patients who drink enough to avoid volume depletion and consequent fall in GFR (111).

Dilutional Acidosis

The rapid expansion of ECF volume with fluids that do not contain HCO_3^- leads to a dilution of HCO_3^- and mild metabolic acidosis. The fall in HCO_3^- produced in this manner is typically quite small (e.g., 10%) and usually is corrected fairly rapidly by renal generation of HCO_3^- (i.e., by renal correction) (112). This is also expected in the setting of therapeutic plasma exchange where large amounts of albumin are rapidly administered (113).

Addition of HCl

Administration of HCl or congeners (e.g., ammonium chloride or lysine chloride) rapidly consumes an HCO_3^- molecule without generating an organic anion, thus causing hyperchloremic metabolic acidosis (114).

Parenteral Alimentation

Amino acid infusions without concomitant administration of alkali (or alkali-generating precursors) may produce hyperchloremic metabolic acidosis in a manner similar to addition of HCl. This problem can be avoided by replacing the chloride salt of these amino acids with an acetate salt, which is metabolized to HCO_3^- (115).

Sulfur Ingestion

Ingested elemental sulfur or sulfur released during metabolism of sulfur-containing amino acids (e.g., methionine or cysteine) is oxidized to sulfate with accompanying H^+ production. Sulfate is excreted rapidly by the kidneys, usually accompanied by sodium, whereas the excretion of H^+ produced by sulfur metabolism lags, resulting in a hyperchloremic metabolic acidosis. A dietary intake rich in sodium compared to potassium and excessive consumption of sulfur-containing amino acids is a common feature of Western diets. Ingestion of 40 to 50 g/day of flowers of sulfur for several days, a folk remedy for constipation, has also produced profound hyperchloremic metabolic acidosis (116).

CAUSES OF INCREASED ANION GAP (ORGANIC METABOLIC ACIDOSIS)

Organic Acidosis Resulting from Increased Acid Production

Lactic Acidosis

Lactic acidosis is an extensively studied organic acidosis. Causes of lactic acidosis are summarized in Table 3-4. Lactic acid is the final product of mammalian anaerobic metabolism. In general, aerobic tissues metabolize carbohydrates to pyruvate, which then undergoes oxidative metabolism within mitochondria. This oxidative metabolism regenerates nicotinamide adenine dinucleotide $(NAD)^+$ consumed at a more proximal site in the glycolytic pathway. When tissues must perform anaerobic glycolysis to regenerate this NAD^+, the net effect is to generate lactic acid from carbohydrates and thus generate H^+. Under normal conditions in humans, relatively small amounts of lactate, specifically the L-isomer, are formed during normal metabolism and are metabolized by the liver, maintaining relatively low plasma and urine levels of this metabolite. Lactic acidosis may develop under pathologic conditions associated with either local or systemic decreases in oxygen delivery (type A), impairments in oxidative

metabolism (type B), or impaired hepatic clearance (117,118).

The diagnosis of lactic acidosis must be considered in all forms of metabolic acidosis associated with an increased anion gap, particularly those cases occurring in these clinical circumstances. Determination of the serum or plasma lactate level may confirm this diagnosis, although many clinical laboratories may not provide this information on an emergency basis (112). In cases of D-lactic acidosis (e.g., seen with blind intestinal loops colonized with D-lactate–producing organisms), the usual measurement of lactate performed in clinical laboratories using an enzymatic reaction does not detect this D-isomer. Nonroutine measurement techniques such as ^{1}H NMR spectroscopy (which does not distinguish between D and L forms) or specific measurement of the D form with the appropriate enzymatic analysis may be necessary to document elevations of D-lactate in this unusual clinical circumstance (119,120).

Treatment of lactic acidosis must be directed at the underlying pathophysiology. Although the degree of acidemia in this setting may become deleterious in its own right, therapy with $NaHCO_3^-$ to directly address the metabolic acidosis has not been found to be effective clinically (121) and is actually deleterious in several experimental models (50,122–124). This issue remains quite controversial at this time (125).

Diabetic Ketoacidosis

DKA results from insufficient insulin to metabolize glucose and excess glucagon, which generates short-chain fatty ketoacids, specifically β-hydroxybutyric and acetoacetic acids. These ketoacids are both relatively strong acids that dissociate almost completely at physiologic pH into H^+ and the keto-anions and cause an anion gap metabolic acidosis. Interestingly, the amount of insulin needed for catabolism of short-chain fatty acids is significantly less than that necessary for glucose homeostasis. Thus, DKA is a common presentation in patients with insulin-dependent diabetes mellitus (111). However, DKA also occurs in patients with non–-insulin-dependent diabetes mellitus (126). In addition, patients with non–insulin-dependent diabetes mellitus may present with marked increases in serum glucose concentrations without ketosis (127) (e.g., nonketotic hyperglycemic hyperosmolar coma). A newer class of hypoglycemic agents, the sodium–glucose cotransporter inhibitor, may predispose to ketone bodies generation and acidosis, especially at time of stress with relatively low glucose concentration (128).

Table 3–4 Causes of Lactic Acidosis

Primary Decrease in Tissue Oxygenation
Septic shock
Cardiogenic shock
Hypovolemic shock
Mesenteric ischemia
Hypoxemia
Excessive Energy Expenditures
Seizures
Extreme exertion
Hyperthermia
Deranged Oxidative Metabolism
Diabetic ketoacidosis
Malignancy
Intoxication (e.g., ethanol, iron, isoniazid, carbon monoxide, strychnine)
Impaired Lactate Clearance
Liver failure
Miscellaneous
D-Lactic acidosis

Patients with DKA typically present with altered sensorium, deep respirations, and severe anion gap metabolic acidosis, with HCO_3^- concentrations as low as 1 to 10 mmol/L and arterial pH values that may be <7.0. Initially, the increase in the anion gap above normal may parallel the decrease in HCO_3^-; however, during therapy, a dissociation of the decrease in anion gap (ΔAG) and the decrease in HCO_3^- concentration ($\Delta[HCO_3^-]$) may develop. This is because of renal elimination of the ketoacidosis as renal perfusion and clearance improve during therapy of the ketoacids; also, it may signify a degree of underlying distal RTA (e.g., hyporeninemic hypoaldosteronism) in some subjects (129).

The diagnosis of DKA is made by finding the combination of anion gap metabolic acidosis, hyperglycemia, and demonstration of serum (or urine) ketoacids. We stress that the presence of serum and urine ketones is

not specific for DKA, but also may be present in other conditions such as alcoholic ketoacidosis (AKA) and starvation ketoacidosis (110) as well as some drug intoxications (e.g., salicylate, ketamine) (130,131).

Some patients who present with DKA may be critically ill; however, mortality is quite low with appropriate therapy. Insulin, hydration, and management of electrolyte disturbances are the essentials of therapy. Most patients with DKA present with considerable total body deficits of potassium, magnesium, and phosphorus, even though serum levels (particularly of potassium) may be high on presentation (132). Treatment of DKA with $NaHCO_3$ still has some proponents despite absence of evidence to support its use (133,134). Hazards associated with $NaHCO_3$ in this setting include hyperosmolarity (ampoules of $NaHCO_3$ are quite hypertonic), overshoot alkalosis, and paradoxical intracellular acidosis (discussed later in the text), which may further compromise CNS function and hemodynamic stability (133). Thus, we do not recommend $NaHCO_3$ treatment for DKA.

Starvation

Voluntary or involuntary abstinence from caloric intake produces relative insulin deficiency and glucagon excess, which is similar to the hormonal disturbances seen with DKA. Specifically, during starvation, hepatic ketogenesis is accelerated and tissue ketone metabolism is reduced. Thus, an increase in the plasma and urine concentrations of ketoacids occurs. Moreover, with prolonged starvation, decreases in plasma HCO_3^- may also transpire, producing a mild anion gap metabolic acidosis. However, the plasma [HCO_3^-] rarely falls to values <18 mmol/L in this setting. Ketone bodies stimulate pancreatic cells to release insulin, and lipolysis is controlled resulting in much less severe ketoacidosis as compared to patients with frank DKA (135).

Alcoholic Ketoacidosis

AKA is probably the result of the combination of alcohol toxicity and starvation. Serum glucose levels range from very low (i.e., <50 mg/dL) to modestly elevated (e.g., 250–275 mg/dL), where confusion with the diagnosis of DKA may occur. Typically, patients present not with simple metabolic acidosis but rather with a complex acid–base disturbance containing features of anion gap metabolic acidosis, metabolic alkalosis

produced by vomiting, and respiratory alkalosis owing to hyperventilation. A markedly increased anion gap is a hallmark of this disorder. Rarely devastating complications such as sudden cardiac death occur (136).

At times, this diagnosis may not be easy to make. This is because when the pH is low, the majority of ketoanions circulating in the serum may not be detected by the Acetest assay. Specifically, the Acetest reaction measures acetoacetate but is rather insensitive to β-hydroxybutyrate. When the serum pH is low, as might be the case with severe AKA, the proportion of β-hydroxybutyrate increases secondary to an increase in the $NADH/NAD^+$ ratio because of alcohol metabolism. Proton NMR spectroscopy of the urine has been used to identify AKA as well as other causes of anion gap metabolic acidosis (119,137). Treatment of AKA consists of vigorous volume and glucose repletion with additional attention to repletion of potassium, magnesium, phosphorus, and vitamin deficits (138).

Nonketotic Hyperosmolar Coma

Some patients with nonketotic hyperosmolar coma with severe hyperglycemia also may present with anion gap metabolic acidosis. In most cases, the organic anion that accumulates has not been identified but does not appear to be either ketoanions or lactate (139,140).

Inborn Errors of Metabolism

The accumulation of organic acids in body fluids with a resultant metabolic acidosis may be seen in certain inborn errors of metabolism, such as maple syrup urine disease, methylmalonic aciduria, propionic acidemia, and isovaleric acidemia. These disorders generally present shortly after birth (141).

Toxic Alcohol Ingestion

Important causes of anion gap metabolic acidosis are those because of toxic alcohol ingestion, specifically methanol and ethylene glycol. Early diagnosis allows for prompt and usually successful therapy, whereas delay in the diagnosis may be associated with considerable morbidity and mortality. Patients who ingest either methanol or ethylene glycol generally develop profound anion gap metabolic acidosis during the course of their illness, but their acid–base status initially may be normal if they present

soon after ingestion. The serum osmolal gap generally is elevated soon after ingestion because of the presence of toxic alcohol in the serum (142–144).

$$\text{Calculated serum osmolality} = 1.86\,[Na^+] + [\text{glucose}]/18 + [\text{urea nitrogen}]/2.8 \quad (3.13)$$

([Na^+] in mM, the [glucose] and [urea nitrogen] in mg/dL)

$$\text{Serum osmolal gap} = \text{measured serum osmolality} - \text{calculated serum osmolality} \quad (3.14)$$

The osmolal gap tends to collapse further along the course of this disease, whereas anion gap metabolic acidosis worsens. Although useful in suggesting this diagnosis, elevations in the serum osmolar gap are not specific for toxic alcohol ingestion, largely because ethanol is the most common cause of an elevated serum osmolal gap (145,146).

Patients who ingest methanol either as a suicide attempt or accidentally typically present with abdominal pain, vomiting, headache, and visual disturbances. Methanol intoxication characteristically produces severe retinitis, which may lead to blindness, and may be detectable on funduscopic examination. Methanol toxicity generally is believed to result from the metabolism of the methanol by alcohol dehydrogenase, specifically to formic acid. Ingestions of as little as 30 mL of methanol are toxic, and 100 to 250 mL of methanol generally is fatal unless treated (144).

Ethylene glycol is the major osmolyte in most commercial antifreeze formulations. Ingestion may occur as a suicide attempt, but because of its sweet taste, accidental ingestions are quite common. Ethylene glycol intoxication is similar to that of methanol in that both produce CNS disturbances and severe anion gap metabolic acidosis. In contrast to methanol, ethylene glycol does not usually produce retinitis, but can cause acute and chronic renal failure. The major toxicity of ethylene glycol is caused by its metabolism by alcohol dehydrogenase to glycolate, glyoxylate, and oxalate (147). Detection of oxalate crystals in the urine may support the clinical impression of ethylene glycol ingestion. Renal biopsy in this setting is characterized by the deposition of calcium oxalate crystals in the tubular epithelial cells and areas of acute tubular necrosis (148). The lethal dose of ethylene glycol is believed to be as little as 100 mL (142).

Because metabolism of methanol and ethylene glycol directly leads to their major toxicities, immediate prevention of this metabolism plays an important role in the therapy of these intoxications. Fortunately, the affinity of alcohol dehydrogenase for ethanol is considerably greater than

for either methanol or ethylene glycol, and infusing ethanol to achieve concentrations >100 mg/dL effectively prevents alcohol dehydrogenase–-mediated metabolism of both ethylene glycol and methanol. Hemodialysis is an effective procedure to facilitate clearance of the nontoxic parent compounds. However, during hemodialysis, adjustments in the dosage of ethanol (which also is cleared by hemodialysis) are necessary to maintain sufficient blood concentrations (149). Fomepizole, a specific inhibitor of alcohol dehydrogenase that has been used in veterinary medicine for some time, has been approved by the Food and Drug Administration for the treatment of methanol and ethylene glycol intoxications (150).

Isopropyl alcohol, another toxic alcohol that is associated with an osmolar gap, is metabolized to acetone, which does not cause metabolic acidosis nor rise in SAG. Generally speaking, the presence of metabolic acidosis is used to rule out isopropyl alcohol ingestion. The use of fomepizole is contraindicated in the setting of isopropyl alcohol intoxication because it reduces the clearance of isopropyl alcohol and prolongs its effect. Clinicians are advised that isopropyl alcohol intoxication combined with alcohol ketoacidosis can be misdiagnosed as methanol or ethylene glycol intoxication, which can result in the inappropriate administration of fomepizole (151).

Salicylate Overdose

Ingestion of large amounts of aspirin, salicylamide, bismuth salicylate, or methyl salicylate may lead to serious and complex acid–base abnormalities. Symptoms correlate quite poorly with blood levels, especially in elderly persons, but almost always accompany extremely elevated blood levels (plasma [salicylate] >50 mg/dL) (123). Salicylates have a CNS effect to stimulate respiration and produce a component of respiratory alkalosis, especially early in the course of toxicity. Most adults with salicylate toxicity present either with respiratory alkalosis or with mixed anion gap metabolic acidosis and respiratory alkalosis. In children, the decreases in plasma [HCO_3^-] and increases in the anion gap develop more rapidly, and presentation with simple anion gap metabolic acidosis is most common. The acids responsible for the metabolic acidosis and increase in the anion gap include salicylate itself as well as endogenous acid anions whose metabolism is affected by the toxic amounts of salicylates. A blood salicylate concentration of 100 mg/dL contributes 7.3 mEq/L to the anion gap. Some component of lactic acidosis generally accompanies severe salicylate toxicity (152).

The diagnosis of salicylate toxicity is suggested by the history of aspirin use, nausea, and tinnitus. This is further supported by the clinical findings of unexplained hyperventilation, anion gap metabolic acidosis, noncardiogenic pulmonary edema, and an elevated prothrombin time. When salicylate toxicity occurs in younger adults, it is generally a result of a suicide attempt and is easily diagnosed; however, the diagnosis may be more elusive in older adults as well as in children. Delayed toxicities have been reported with the use of enteric coated aspirin, salicylate-induced pylorospasm, or the formation of pharmacobezoars. Therefore, it is imperative that salicylate concentrations are monitored every 4 hours until the levels are decreasing and the patient is not symptomatic (153). Advanced age and a delay in the diagnosis of salicylate toxicity are associated with significant mortality.

Treatment of salicylate toxicity generally should include alkalization of the blood and urine with sodium bicarbonate. Despite the potential negatives associated with sodium bicarbonate use in acute anion gap metabolic acidosis, alkalization of the plasma decreases the diffusion of salicylate into CNS sites where it is toxic, and it improves renal excretion as the urine is alkalinized (154). However, hemodynamic compromise and fluid overload must be carefully avoided, especially in older patients or those with underlying heart disease (155). Sustained low-efficiency dialysis has been shown to be quite effective at removing salicylate from the body and should be considered for patients with severely elevated plasma levels (>90 mg/dL), or evidence of severe toxicity, or for patients for whom aggressive alkalization may be hazardous (155).

Other Intoxications

A variety of other agents may produce anion gap metabolic acidosis. These include strychnine, oral iron overdose, isoniazid, papaverine, outdated tetracyclines, hydrogen sulfide, carbon monoxide, and paraldehyde. In general, these produce lactic acidosis (150). Severe lactic acidosis has also been reported with the daily ingestion of mangosteen, a tropical fruit used for weight loss (156). Sedatives used in critical care setting such as propofol and lorazepam can also cause lactic acidosis because of the propylene glycol solvent (157,158). Cyanide poisoning may dramatically impair mitochondrial function, with large increases in lactic acid production resulting in anion gap metabolic acidosis (159).

Other agents that may cause nonlactate anion gap metabolic acidosis include acetaminophen, toluene, and citric acid. Acetaminophen in

therapeutic doses may generate pyroglutamic acid (5-oxoproline) in susceptible individuals. Bicarbonate levels as low as 3 mM and anion gaps >35 mEq/L have been seen, and repeated episodes may occur in the same patient (160,161). No metabolic defect has been identified. Toluene, which produces RTA (generally distal, but may include a proximal component), causes an elevation of serum hippurate (a metabolite of the toluene) concentration (162). The hippurate is excreted rapidly if renal function is intact and hyperchloremic metabolic acidosis may ensue. Another exception is citric acid present in toilet bowl cleaner, which increases the anion gap and causes hyperkalemia. Administration of intravenous calcium was necessary to stabilize a reported patient (163).

Failure of Acid Excretion

Acute or Chronic Renal Failure

The failure of the kidney to excrete the usual 1 to 3 mmol/kg of acid produced each day leads to metabolic acidosis. With both acute and chronic renal failure, some retention of anions occurs (including phosphate, sulfate, and some poorly characterized organic anions), which produces some increase in the SAG (164). Metabolic acidosis in the setting of acute and chronic renal failure generally is not severe unless a markedly catabolic state occurs or another acidotic condition supervenes.

With acute renal failure, the sudden loss of renal excretory function is invariably accompanied by a failure of acid excretion. Adaptation has no time to occur in this setting. With chronic renal failure, adaptation in remaining nephrons has time to occur. Specifically, the remaining nephron units markedly increase their NH_4^+ excretion. Metabolic acidosis is caused by a failure of the enhanced ammonia genesis in remaining nephron units to achieve the necessary NAE required for acid–base balance. Phosphate retention, which ultimately decreases urinary phosphate involved in the titratable acid component of NAE, also may contribute to this failure of acid–base balance. In addition, the high concentrations of circulating PTH seen in chronic renal failure decrease proximal tubular HCO_3^- reabsorption and participate in the pathogenesis of metabolic acidosis (165). Although therapy of the metabolic acidosis with supplemental sodium bicarbonate may be useful in some cases of chronic renal failure, generally, the development of metabolic acidosis is accompanied by other manifestations of chronic renal failure that mandate institution of dialysis or renal transplantation.

TREATMENT OF METABOLIC ACIDOSIS

Although acidosis itself is deleterious to the function of many organs, the treatment of most conditions associated with metabolic acidosis generally is best accomplished by treatment of the underlying disease state. With most of the hyperchloremic states of metabolic acidosis, gradual correction of the acidosis with HCO_3^- administered either as sodium bicarbonate or as a substrate metabolized to HCO_3^- (e.g., citrate) is quite rational, effective, and ultimately beneficial. Oral administration of these agents is preferred. In general, 1 g of sodium bicarbonate delivers about 2 mmol of HCO_3^-. Commercially available sodium or mixed sodium and potassium citrate solutions (e.g., Shohl's solution or Polycitra) contain 1 mmol of HCO_3^- equivalent per milliliter. Although citrate solutions are generally better tolerated than sodium bicarbonate tablets (which cause bloating when they produce CO_2 gas in the stomach), citrate may increase GI absorption of aluminum and should not be administered along with aluminum-based phosphate binders, especially in the setting of chronic renal failure (166).

The acute treatment of metabolic acidosis associated with an increased anion gap with intravenous sodium bicarbonate actually may be deleterious, especially in conditions associated with impaired tissue perfusion. To understand how acute therapy with sodium bicarbonate may be deleterious, one must consider the fate of the administered HCO_3^- molecules. When sodium bicarbonate is given, a change in the serum HCO_3^- concentration results. The magnitude of this change in serum HCO_3^- for a given dose of bicarbonate is determined by the apparent volume of distribution for HCO_3^- ($Vd_{HCO_3^-}$), which we define as

$$Vd_{HCO_3^-} = \text{dose of } HCO_3^- / (\Delta \text{ serum}[HCO_3^-]) \qquad (3.15)$$

This $Vd_{HCO_3^-}$ is not constant, but increases with increasing severity of acidosis. This variation in $Vd_{HCO_3^-}$ probably results both from increased buffering from some extracellular and intracellular proteins and from alterations in pHi homeostasis. The addition of HCO_3^- to blood (or an organism) produces CO_2 by mass action. Again, when metabolic acidosis is present, more CO_2 is produced for a given dose of sodium bicarbonate. In fact, recent studies performed in a closed, human blood model

demonstrate that the production of CO_2 from administered HCO_3^- is directly dependent on the initial pH. Therefore, when ventilation is normal, this extra CO_2 is rapidly eliminated by the lungs, and a portion of the $Vd_{HCO_3^-}$ can be considered to be extracorporeal. However, when pulmonary ventilation or, more commonly, tissue ventilation is impaired (by poor tissue perfusion), this CO_2 generated by infused HCO_3^- may diffuse into cells (far more rapidly than the original HCO_3^- molecule) and paradoxically decrease the pHi. This is shown schematically in Figure 3-3. Experimentally, administration of sodium bicarbonate in animal models of metabolic acidosis has been associated with a fall in pH in several organs as well as additional hemodynamic compromise. In addition to this paradoxical intracellular acidosis, administration of hypertonic sodium bicarbonate (often given as 50 mL ampoules of 1 mol/L $NaHCO_3$) may be associated with development of hypertonicity as well. This hypertonicity itself may have deleterious effects on cardiac function, especially in the setting of cardiac arrest resuscitation (167). In general, we do not advocate the emergency administration of intravenous sodium bicarbonate for acute anion gap metabolic acidosis, although this area remains quite controversial (134). Some small studies suggest that administering large amounts of bicarbonate while providing continuous hemodialysis and ultrafiltration may be a useful approach in this setting (168,169); however, this approach has not been subjected to rigorous study and remains speculative.

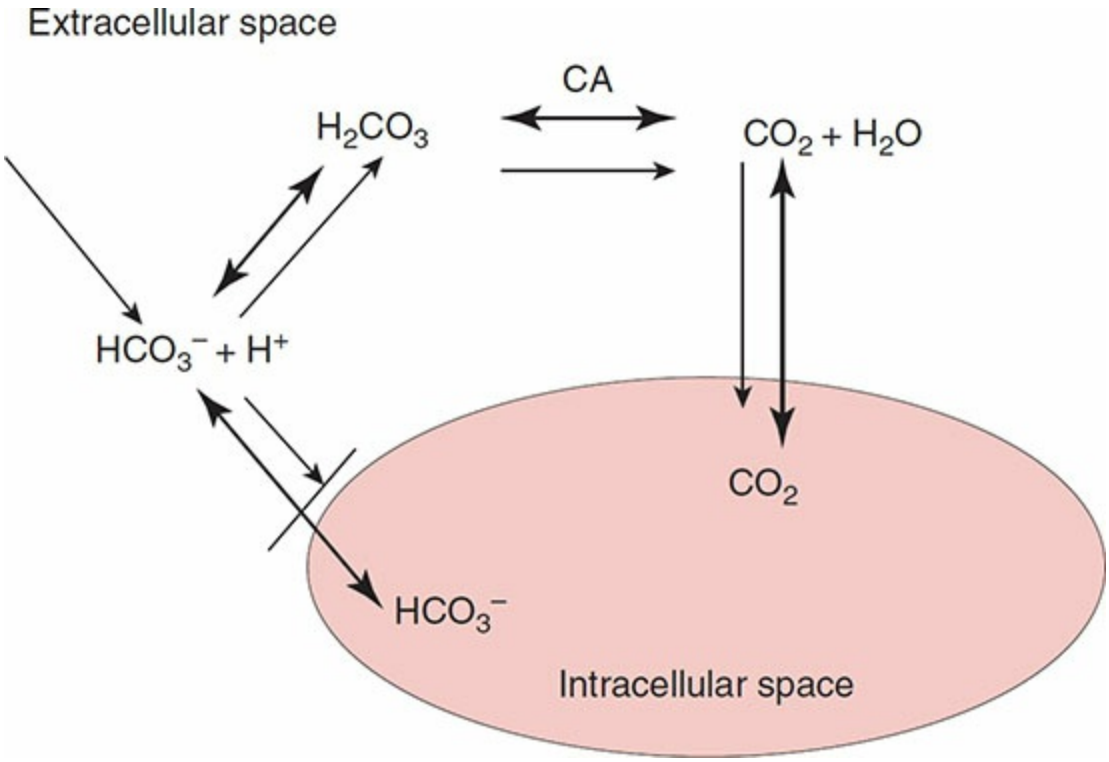

Figure 3–3 Illustration of mechanism underlying paradoxical intracellular acidosis resulting from sodium bicarbonate administration. When additional HCO_{3-} is added to the extracellular fluid (*narrow arrow*), HCO_{3-} combines with H^+ and reaction is shifted by mass action to H_2CO_3, which results in increases in extracellular CO_2 tension. Because most cell membranes are more permeable to CO_2 than to HCO_{3-}, this transiently causes the cellular CO_2 tension to rise more than the $[HCO_{3-}]$, which results in decreases in intracellular pH.

To address the concerns for sodium bicarbonate discussed in the preceding, alternatives have been proposed. These alternatives include non–CO_2-generating buffers such as THAM (tris-hydroxymethyl aminomethane) (161) and Carbicarb (a 1:1 mixture of disodium carbonate and sodium bicarbonate) as well as dichloroacetate, an agent that decreases lactate production by stimulating the activity of the pyruvate dehydrogenase complex (170). Although all three of the agents have been studied for some time, none have made it into routine clinical practice.

Metabolic Alkalosis

DEFINITIONS AND CAUSES

Metabolic alkalosis is a systemic disorder caused by a process that leads to

an increased pH caused by a primary increase in the plasma [HCO_3^-]. This primary elevation of plasma [HCO_3^-] may be caused by three processes (171).

Net Loss of H*1* from the Extracellular Fluid

H^+ can be lost from the ECF externally through the GI tract and kidneys or be shifted (at least theoretically) internally into cells. If H^+ losses exceed the daily H^+ load produced by diet and metabolism, then increases in the plasma [HCO_3^-] will occur. This is because the loss of H^+ at these sites mandates the generation of an HCO_3^- molecule. In the stomach, the gastric parietal cell secretion of H^+ by the luminal H^+ ATPase leaves an HCO_3^- to be reclaimed at the basolateral surface. In the kidney, H^+ secretion via the H^+ ATPase or Na^+/H^+ exchanger also leaves an HCO_3^- molecule for reclamation on the basolateral surface. Shifting of H^+ into cells has been postulated to accompany states of significant potassium depletion, thus increasing ECF [HCO_3^-]. Despite the appeal of this concept, evidence of intracellular acidosis developing during experimental potassium depletion has not been observed consistently (172).

Net Addition of Bicarbonate Precursors to the Extracellular Fluid

The administration of HCO_3^- or substances that generate HCO_3^-, such as lactate, citrate, or acetate, at a rate greater than that of metabolic H^+ production also leads to a rise in ECF [HCO_3^-]. When renal function is normal, such increases in ECF [HCO_3^-] are offset by marked increases in renal HCO_3^- excretion because the plasma [HCO_3^-] exceeds the PT for HCO_3^- reabsorption.

External Loss of Fluid Containing Chloride in Greater Concentration and Bicarbonate in Lesser Concentration than the Plasma

Loss of this type of fluid leads to both a contraction of the ECF volume

and a rise in [HCO_3^-]. In this situation, H^+ is not lost externally, as in vomiting or nasogastric suction, but rather the remaining ECF [HCO_3^-] increases as ECF volume contracts (contraction alkalosis) (112). However, intrarenal mechanisms to generate and reabsorb HCO_3^- also are active in this state.

PATHOPHYSIOLOGY OF METABOLIC ALKALOSIS

The normal kidney has a wonderful protective mechanism against the development of significant increases in ECF [HCO_3^-], namely, a threshold for tubular fluid [HCO_3^-] above which proximal reabsorption falls and HCO_3^- losses in the urine ensue. Therefore, in virtually all cases of metabolic alkalosis, the kidney must participate in the pathophysiology, at least at a passive level, by not excreting the excess HCO_3^-. A useful way to consider the pathogenesis of metabolic alkalosis is to separate factors that initiate or generate the metabolic alkalosis from those that maintain it.

Buffering

When HCO_3^- is added to the ECF, H^+ reacts with the HCO_3^- to produce CO_2, which normally is exhaled in expired gas. Thus, the increase in plasma and ECF [HCO_3^-] is attenuated. Most of the H^+ used in this buffering comes from the ICF and from a small increase in lactic acid production (118).

Respiratory Compensation

The control of ventilation under normal conditions apparently is situated in the brain stem and is most sensitive to interstitial H^+ concentrations. The respiratory compensation to metabolic alkalosis follows the same principles as respiratory compensation to metabolic acidosis. However, the direction of the change of P_{CO_2} is different. Hypercapnia caused by hypoventilation rather than hypocapnia caused by hyperventilation occurs. Constraints regarding oxygenation limit the magnitude of this hypoventilatory response. As a rule of thumb, the Pa_{CO_2} should increase 0.25 to 1.0× the increase in plasma [HCO_3^-] during metabolic alkalosis.

Failure to demonstrate such compensation in the setting of metabolic alkalosis marks the coexistence of primary respiratory alkalosis (173).

Renal Correction

The response by the kidney to excrete excessive HCO_3^- in the urine, under normal conditions, rapidly corrects metabolic alkalosis. In a manner analogous to tubular reabsorption of glucose, one can consider the maximal amount of tubular bicarbonate reabsorption (T_{max}) as well as the PT above which bicarbonaturia occurs. Bicarbonate excretion in the urine is proportional to the GFR once the PT is exceeded. Therefore, if a patient has normal renal function, it is difficult to produce a sustained increase in plasma [HCO_3^-] without somehow changing the PT and T_{max}.

Biochemical and Physiologic Responses

FACTORS IN THE MAINTENANCE OF METABOLIC ALKALOSIS

Several factors tend to increase the apparent T_{max} for HCO_3^- and thus increase net HCO_3^- reabsorption by the kidney.

Decreases in Effective Arterial Blood Volume

Absolute (e.g., salt losses through vomiting or bleeding) or effective (e.g., heart failure, cirrhosis, nephrotic syndrome) arterial volume depletion increases the T_{max} and PT for HCO_3^-. This is accomplished both proximally (increased proximal tubule reabsorption of Na and water) and distally (mineralocorticoid effect) in the nephron (174).

Chloride Depletion

Although chloride depletion occurs as part of the pathophysiology of decreases in ECF volume, detailed physiologic studies show that the chloride anion is independently involved in the process of HCO_3^- reabsorption. Specifically, even in the presence of expansion of the ECF, depletion of chloride leads to increases in the apparent T_{max} and PT for

HCO_3^- (175).

Aldosterone

An increase in distal sodium avidity resulting in increased renal HCO_3^- generation may occur in the absence of decreases in effective arterial blood volume if mineralocorticoids are administered or produced locally (176).

Potassium Depletion

Potassium depletion is another factor implicated in increasing the apparent T_{max} and PT for HCO_3^- and maintaining metabolic alkalosis. One explanation is that potassium depletion leads to a relative intracellular acidosis, and this relative intracellular acidosis makes renal H^+ excretion more favorable (177). Evidence against this concept includes the considerable concentration differences involved; that is, it is difficult to evoke the argument of preservation of electroneutrality by an ion that exists in nM concentrations. In addition, investigators have failed to detect a decrease in renal pHi with ^{31}P NMR spectroscopy during potassium depletion (178). Moreover, in human studies, metabolic alkalosis can be corrected almost completely without correction of potassium depletion (179).

Hypercapnia

Increases in $PaCO_2$ are known to increase the apparent T_{max} and PT for HCO_3^-. This may be mediated through decreases in cellular pHi (which have been documented during acute and chronic hypercapnia). Interestingly, the increases in $PaCO_2$ that occur during metabolic alkalosis as part of normal respiratory compensation actually tend to impair renal correction through this mechanism (180).

CLINICAL FEATURES

No symptoms or signs are specific for metabolic alkalosis. The disturbance should be suspected, however, in patients who have muscle cramps, weakness, arrhythmias, or seizures, especially if the appropriate clinical

scenario (e.g., diuretic use, vomiting) is present. Some of these signs and symptoms may be related to alterations in ionized calcium because increases in pH cause plasma proteins to bind calcium more avidly, thus lowering ionized calcium concentrations. Severe alkalemia (pH > 7.6) may be associated with malignant arrhythmias as well as seizures (171).

LABORATORY FINDINGS IN METABOLIC ALKALOSIS

Arterial blood gases reveal the diagnostic pattern, that is, an increased pH, increased [HCO_3^-], and an increased $PaCO_2$, with the increase in $PaCO_2$ being between 0.25 and 1× the increase in [HCO_3^-]. The serum electrolytes demonstrate increased TCO_2 as well as decreased chloride and, usually, diminished potassium concentrations. The hypokalemia results from both shifting of potassium into cells and increased renal losses. Potassium shifts into cells during both respiratory and metabolic alkalosis; however, the magnitude of such changes is difficult to predict. Renal losses are enhanced throughout the course of metabolic alkalosis. The SAG may be increased by up to 9 to 12 mEq/L with severe metabolic alkalosis. This is owing to some small increases in lactate concentrations (118), but mostly to the increased electronegativity of albumin with elevated pH (181).

Urine chemistries represent an important step in the classification of metabolic alkalosis. Specifically, urine electrolytes are used to determine whether decreases in effective arterial blood volume act as a maintenance factor in the pathogenesis of metabolic alkalosis. Although the urine sodium concentration may be inconsistent in this condition, especially if bicarbonaturia is present at the time the urine sample is collected, the urine chloride concentration allows one to classify patients into chloride-responsive and chloride-unresponsive categories of metabolic alkalosis. Chloride-responsive metabolic alkalosis corrects when volume expansion or improvement of hemodynamics occurs. Examples include patients who are vomiting or have received diuretics, but have discontinued them. Chloride-unresponsive metabolic alkalosis does not correct with these maneuvers. Examples are patients with primary mineralocorticoid excess or patients who are continuing to take diuretics. In patients with chloride-responsive metabolic alkalosis, the urine chloride concentration is <10 mmol/L, whereas patients with chloride-unresponsive metabolic alkalosis have a urine chloride concentration >20 mmol/L.

DIFFERENTIAL DIAGNOSIS OF METABOLIC ALKALOSIS

The differential diagnosis of metabolic alkalosis generally is approached by separating patients into those who have chloride depletion as a maintenance factor (chloride responsive), those who do not have chloride depletion as a maintenance factor (chloride unresponsive), and those who have an unclassified (generally uncommon) form of metabolic alkalosis. As discussed, this generally is accomplished using the urine chloride concentration (Table 3-5).

Chloride-responsive Metabolic Alkalosis

Vomiting

Gastric secretory volume may exceed 1 to 2 L/day in patients with persistent vomiting. The gastric secretions may contain as much as 100 mmol/L of H^+, and because the gastric parietal cells generate an HCO_3^- molecule for each H^+ secreted, as much as 200 mmol of HCO_3^- may be generated in 1 day. Although this represents a significant initiation factor, it must be stressed that the concomitant Na^+ and Cl^- losses (as much as 400 mmol/day), possibly along with the associated K^+ losses (more in the urine than in vomit, which generally has <15 mmol/L potassium), are the maintenance factors that allow metabolic alkalosis to be maintained (182). This is shown schematically in Figure 3-4.

Table 3–5 Differential Diagnosis of Metabolic Alkalosis

Chloride-responsive Metabolic Alkalosis
Vomiting
Gastric drainage
Villous adenoma
Chloride diarrhea
Diuretics
Posthypercapnia
Cystic fibrosis
Chloride-resistant Metabolic Alkalosis
Hyperaldosteronism
Cushing syndrome
Bartter syndrome

Licorice
Profound potassium depletion?

Unclassified Metabolic Alkalosis

Alkali administration
Milk–alkali syndrome
Transfusion of blood products
Hypercalcemia
Poststarvation
Large doses of penicillin antibiotics

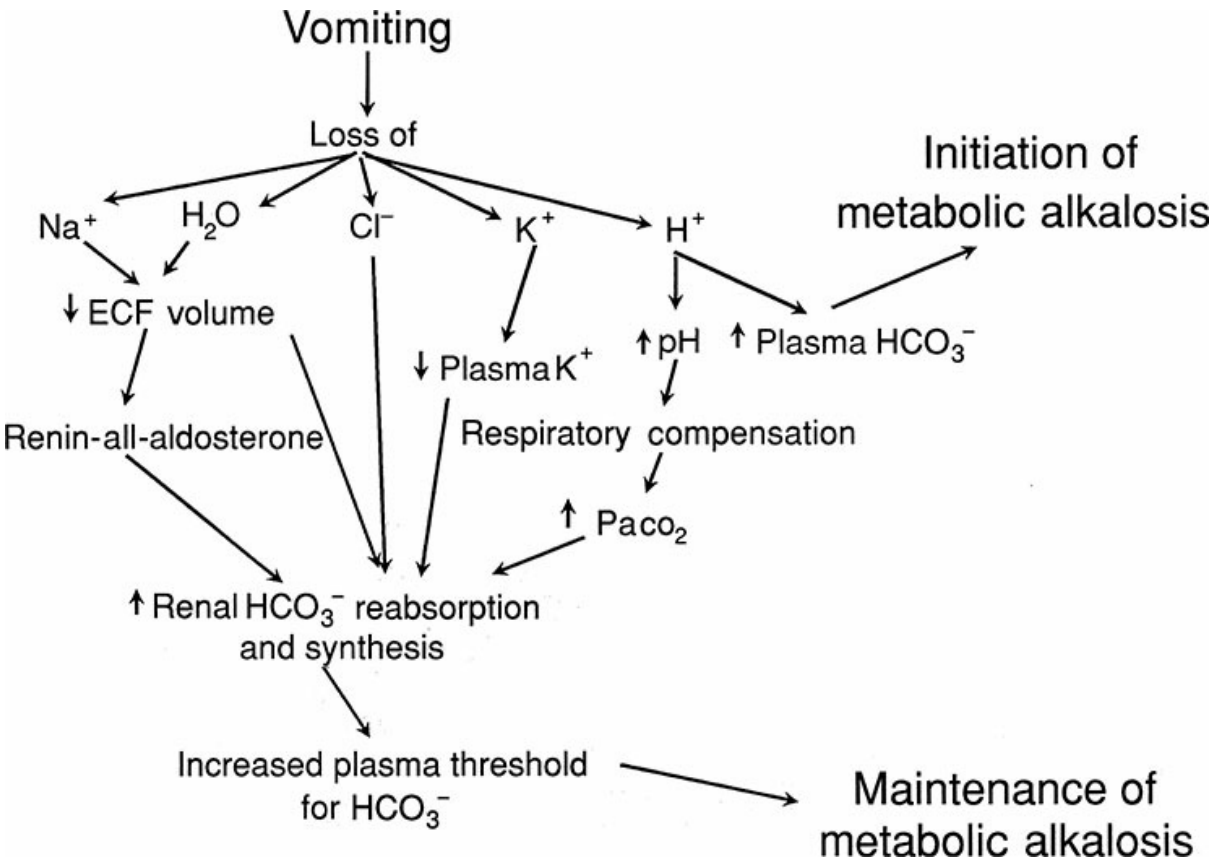

Figure 3–4 Initiation and maintenance factors in the pathogenesis of metabolic alkalosis developing from vomiting are illustrated. Initial loss of H^+ from the stomach is the initiation factor where the concomitant loss of K^+, Na^+, Cl−, and H_2O along with respiratory compensation sets up the maintenance of the alkalosis by increasing renal HCO_{3-} reabsorption and synthesis and the plasma threshold for HCO_{3-}.

The degree of metabolic alkalosis associated with vomiting generally is mild; however, in conditions where gastric secretions are greatly stimulated, such as Zollinger–Ellison syndrome with gastric outlet obstruction, plasma [HCO_3^-] may exceed 60 mmol/L (72).

Gastric Drainage

The pathophysiology of gastric drainage, usually through a nasogastric tube, is identical to that of vomiting (182).

Villous Adenoma of the Colon

Villous adenoma of the colon may result in profound diarrhea, which is extremely rich in protein, sodium, potassium, and chloride. The losses of sodium, potassium, and chloride and the relatively low [HCO_3^-] concentration in the diarrheal fluid in some patients may lead to metabolic alkalosis (183). However, it must be stressed that this condition more commonly leads to sufficient HCO_3^- losses to produce metabolic acidosis, as discussed earlier (72).

Chloride Diarrhea

Chloride diarrhea is a rare congenital syndrome arising from a defect in small and large bowel chloride absorption that leads to a chronic diarrhea with a stool fluid rich in chloride. Metabolic alkalosis develops through the mechanisms described in the preceding for villous adenoma (72).

Diuretic Therapy

Diuretics that exert their effects either in the thick ascending limb of Henle (loop diuretics such as furosemide, bumetanide, torsemide) or in the distal tubule (thiazide diuretics) may facilitate volume depletion as well as directly stimulate renin secretion (possibly through increases in distal tubular fluid sodium content). Thus, they both initiate metabolic alkalosis through H^+ losses and maintain metabolic alkalosis via volume depletion and ongoing H^+ losses by the kidney (if diuretics are continued). If the urine chloride is obtained while diuretic effects persist, it may be high, whereas if the urine chloride is determined after sufficient time has elapsed to eliminate diuretic effects (generally >24–48 hours), it should be low, reflecting volume depletion. Metabolic alkalosis along with hypokalemia is an extremely frequent complication of diuretic use and should suggest the possibility of diuretic use especially in adolescents and adults, even if such drugs are not prescribed. Diuretic abuse is seen commonly in patients suffering from anorexia nervosa (184).

Posthypercapnia

As discussed in Chapter 4, the renal compensation to chronic hypercapnia results in an elevation in plasma [HCO_3^-]. When hypercapnia is rapidly corrected (e.g., with intubation and mechanical ventilation), the patient is left with an elevated plasma [HCO_3^-] until renal correction occurs, a process that generally takes at least several hours. This metabolic alkalosis may persist if sufficient chloride is not provided to allow for renal correction (185). Possibly a mild metabolic alkalosis may be seen during waking hours in patients with sleep apnea.

Cystic Fibrosis

Children with cystic fibrosis have been reported in whom metabolic alkalosis developed because of marked loss of chloride in the sweat, which had relatively little HCO_3^-. The resultant volume depletion maintained the metabolic alkalosis in these patients (186).

Chloride-resistant Metabolic Alkalosis

Primary Hyperaldosteronism

Aldosterone directly stimulates distal nephron H^+ secretion by several mechanisms, some of which are tied to sodium reabsorption and potassium secretion, whereas others appear to be independent of sodium or potassium transport. This increased H^+ secretion leads to either reclamation of filtered HCO_3^- destined for excretion or the generation of new HCO_3^- that is ultimately retained in the ECF. Although the increase in ECF [HCO_3^-] produced by such distal effects results in ECF volume expansion and decreases in proximal tubule reabsorptive capacity for HCO_3^-, distal processes are sufficient to maintain an elevated PT for HCO_3^-. Hence, the clinical features of hypokalemic metabolic alkalosis are produced, often in concert with hypertension associated with ECF volume expansion.

Such primary increase in mineralocorticoids may be caused by an adrenal tumor, which selectively makes aldosterone (Conn syndrome), or by hyperplasia (usually bilateral) of the adrenal cortex. The diagnosis of such a primary mineralocorticoid excess state is dependent on the demonstration that volume expansion is present (e.g., nonstimulatable

plasma renin activity) and that aldosterone secretion is not suppressible by volume expansion (i.e., demonstration that exogenous mineralocorticoids and high-salt diet or acute volume expansion with saline do not suppress plasma aldosterone levels) (187). In some cases, hyperaldosteronism can be suppressed by the pharmacologic administration of glucocorticoids. Recent studies have demonstrated that this glucocorticoid-remediable aldosteronism is caused by a gene duplication fusing regulatory sequences of the steroid 11^{β}-hydroxylase gene to the coding sequences of the aldosterone synthase gene (188).

Cushing Syndrome

Adrenocorticotropic hormone–secreting tumors, primary adrenal cortical tumors, or hyperplasia owing to congenital enzyme deficiencies may increase corticosteroid synthesis. Many corticosteroids (specifically cortisol, deoxycorticosterone, and corticosterone) also may have considerable mineralocorticoid effects and produce hypokalemic metabolic alkalosis, sometimes accompanied by hypertension. Detailed metabolic analysis of the plasma and urine as well as imaging studies may be necessary to arrive at the precise diagnosis (176).

Bartter and Gitelman Syndromes

Bartter syndrome is a rare condition usually presenting in children characterized by hyperreninemia, hyperaldosteronemia in the absence of hypertension, or sodium retention. Histologically, hyperplasia of the juxtaglomerular apparatus is noted, a finding not specific for this diagnosis (189,190). Functionally, the disorder is believed to be caused by a failure of chloride reabsorption in the thick ascending limb of Henle, a disturbance that results in a very high delivery of chloride and sodium to the distal nephron, activation of the renin–angiotensin–aldosterone system, and production of hypokalemic metabolic alkalosis. Although the PGE system has been suggested to participate in this disturbance, and sometimes PGE synthesis inhibitors may be beneficial, the increase in renal PGEs in this disorder is secondary. Elegant genetic studies have demonstrated that the molecular basis of Bartter syndrome can be attributed to one of three abnormalities. Specifically, inherited inactivity of the $NaKCl_2$ transporter, ROMK (renal outermedullary potassium) channel, and a chloride channel, transport proteins that are essential to the function of the medullary thick ascending limb of Henle, each can result in Bartter

syndrome (191–193). These findings suggest that each of these three components is essential for effective thick ascending limb function. A schematic of how these transporters interact is shown in Figure 3-5. A closely related condition, Gitelman syndrome, now is known to be caused by mutations in the thiazide-sensitive NaCl transporter important in distal tubule function (194). Gitelman syndrome may present in adults and is probably more common than Bartter syndrome.

Because both Bartter and Gitelman syndromes mimic diuretic use so closely on a physiologic basis, it may be difficult to separate them from surreptitious diuretic use unless diuretics are specifically screened for in the urine. The genetic syndromes are uncommon and surreptitious diuretic use is much more common and must be considered foremost in adolescents or adults who present with unexplained hypokalemic metabolic alkalosis (184).

Licorice

A major component of “black” licorice, glycyrrhizic acid, may cause a hypokalemic metabolic alkalosis accompanied by hypertension and thus mimic primary hyperaldosteronism. Recent study demonstrates that glycyrrhizic acid actually inhibits 11^{β}-hydroxysteroid dehydrogenase activity and “uncovers” the mineralocorticoid receptor to be stimulated by glucocorticoids that normally circulate in relatively higher concentrations. Some chewing tobacco also contains this substance and can cause a similar presentation (195).

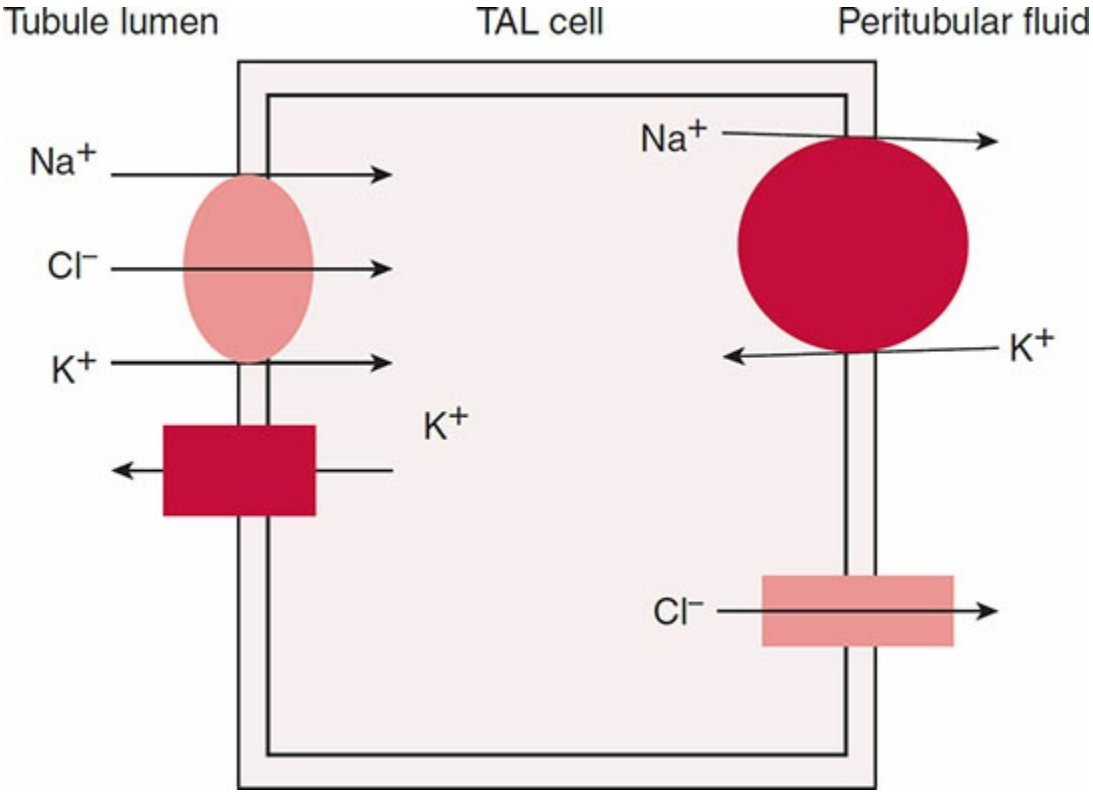

Figure 3–5 Schematic depicting medullary thick ascending limb reabsorption of NaCl from the tubular lumen. Entry of Na^+, K^+, and Cl− occurs through the $NaKCl_2$ transporter (*light-colored ellipse*) with 2 Cl− molecules transported for each Na^+ and K^+. To allow for a continued supply of K^+ in the luminal fluid, K^+ must leak out through the ROMK channel (*dark solid rectangle*). Once Na^+ and Cl− are in the cell, the Cl− must leave through the basolateral side via a chloride channel (*light gray rectangle*), whereas the Na^+ is pumped out by the Na^+/K^+ ATPase (*solid dark circle*).

Profound Potassium Depletion

Several patients with profound hypokalemia (plasma [K^+] <2 mmol/L) have had significant metabolic alkalosis associated with a urine chloride concentration >20 mmol/L without evidence of mineralocorticoid excess. The alkalosis was not corrected during sodium repletion until the potassium deficit also was corrected (177). This suggests that in some cases potassium depletion may convert a chloride-responsive metabolic alkalosis to a chloride-unresponsive metabolic alkalosis. However, correction of metabolic alkalosis without repletion of potassium deficits clearly has been demonstrated in humans (179). Therefore, although potassium supplementation is advisable to correct the potassium deficit and hypokalemia that may contribute to the maintenance of metabolic alkalosis and are problems in their own right, such supplementation does not appear necessary to correct metabolic alkalosis.

Unclassified Metabolic Alkalosis

Alkali Administration

The kidney rapidly excretes alkali, and metabolic alkalosis can be maintained only if a maintenance factor supervenes or the administration of alkali continues. However, in some situations, alkali administration may cause metabolic alkalosis. This alkali load may be in the form of HCO_3^- or organic anions that are metabolized to HCO_3^-, such as citrate or acetate. Specifically, administration of an alkali load may cause sustained metabolic alkalosis in patients with chronic renal failure, where the ability to excrete an HCO_3^- load is impaired because GFR is reduced (196).

Milk–Alkali Syndrome

Milk–alkali syndrome is seen in patients with dyspepsia who consume moderate to large amounts of antacids containing calcium and absorbable alkali (e.g., calcium carbonate). Lack of ECF volume expansion along with hypercalcemia-mediated suppression of PTH secretion contributes to the maintenance of metabolic alkalosis. Hypercalcemia also decreases renal blood flow and glomerular filtration, which can further impair renal correction of metabolic alkalosis. Alkalosis reduces calcium excretion and tends to potentiate associated hypercalcemia. Chronically, nephrocalcinosis may occur, which may ultimately decrease GFR, thus further reducing the ability to excrete an alkali load (197).

Transfusion of Blood Products

Infusion of large amounts (>10 units) of blood products containing the anticoagulant citrate can produce moderate metabolic alkalosis. The production of HCO_3^- from citrate is totally responsible for the initiation of metabolic alkalosis. In other situations, some degree of prerenal azotemia (e.g., when packed red blood cells are given to a patient with hemorrhagic shock) may contribute to the maintenance of the metabolic alkalosis. Patients given parenteral hyperalimentation with excessive amounts of acetate or lactate also may develop metabolic alkalosis through an identical mechanism (196).

Hypercalcemia (Nonhyperparathyroid Etiology)

Mild metabolic alkalosis has been associated with hypercalcemia that results from causes other than hyperparathyroidism (e.g., malignancy, sarcoid). This may be caused by a suppression of PTH that then raises the PT for HCO_3^- (198).

Poststarvation (Refeeding Alkalosis)

Patients who break prolonged fasts with meals containing carbohydrates may develop a metabolic alkalosis that can persist for several weeks. The mechanism for the initiation of the metabolic alkalosis is unknown. Increases in renal sodium avidity (resulting from the ECF volume depletion occurring during starvation) appear to be the maintenance factor (135).

Large Doses of Penicillin Antibiotics

Intravenous administration of large doses of some penicillin antibiotics, specifically penicillin and carbenicillin, may result in hypokalemic metabolic alkalosis. The mechanism is believed to be the increase in delivery of poorly reabsorbable anions to the distal nephron with a resultant increase in H^+ and potassium secretion (199).

TREATMENT OF METABOLIC ALKALOSIS

The guiding principle for treating all acid–base disturbances is to address the underlying disease state. However, in some cases, the degree of acid–base abnormality itself becomes life threatening, especially in mixed acid–base disturbances where the respiratory and metabolic components go in the same direction (e.g., respiratory alkalosis + metabolic alkalosis). When an elevated systemic pH becomes life threatening (e.g., pH > 7.6 with seizures and ventricular arrhythmias), rapid reduction in systemic pH may be accomplished by control of ventilation. In such situations, airway intubation with sedation and controlled hypoventilation with a mechanical ventilator (sometimes using inspired CO_2 and/or supplemental oxygen to prevent hypoxia) may be lifesaving (200).

Although historically administration of either HCl or its congeners (e.g., arginine chloride or ammonium chloride) had been advocated to correct metabolic alkalosis, we do not advocate this approach alone. Our

rationale is that these agents may have significant potential complications (see below). However, of greater importance, they simply do not work fast enough to prevent or treat life-threatening complications. Therefore, we advocate the control of the $PaCO_2$ as outlined in the preceding for urgent intervention. Once the situation is no longer critical, partial or complete correction of the metabolic alkalosis over 6 to 8 hours with HCl administered as a 0.15 mol/L solution through a central vein may be used. Generally, the "acid deficit" is calculated assuming a bicarbonate distribution space of 0.5× the body weight in liters, and about one-half of this amount of HCl is given with frequent monitoring of blood gases and electrolytes. Hemodialysis may be faster and should also be considered, even if other indications for dialysis are not present. We do not advocate ammonium chloride use in this setting because of the risk of ammonia toxicity.

In less urgent settings, therapy of the metabolic alkalosis may be addressed after examining whether it is classified as chloride responsive or not. Chloride-responsive metabolic alkalosis responds quite well to volume repletion and improvement of renal hemodynamics. If hypokalemia is present, it should be corrected as well. Treatment of the chloride-unresponsive metabolic alkalosis conditions generally mandates interference with the mineralocorticoid (or mineralocorticoid-like substance) that is maintaining renal H^+ losses. Sometimes, this can be accomplished pharmacologically with spironolactone or other distal K-sparing diuretics, such as amiloride.

In some cases, the proximate cause of the metabolic alkalosis is necessary for the overall well-being of the patient. One example that comes to mind is a patient with severe heart failure who develops hypokalemic metabolic alkalosis as a result of loop diuretics whose continued use is mandated by the patient's congestive symptoms. In such cases, the proximal diuretic acetazolamide, which decreases the PT for HCO_3^- by inhibiting proximal tubule HCO_3^- reabsorption, may be very effective (101). In subjects who are undergoing persistent gastric drainage, administration of either an H_2 blocker or H^+ ATPase inhibitor to decrease gastric H^+ secretion may be advantageous (201). In patients with advanced chronic renal failure in whom metabolic alkalosis has been induced (e.g., with antacid excess), hemodialysis may be necessary for correction.

General Approach to Acid–Base Diagnosis and Treatment

We propose a relatively simple seven-step method to identify and treat acid–base disturbances. This approach presumes that the clinician has suspected an acid–base disturbance based on history and/or physical examination data or other laboratory data. Once such suspicion exists, a blood gas (which gives pH, O_2, CO_2, and calculated [HCO_3^-] values) and serum chemistry panel (which gives serum Na^+, K^+, Cl^-, and total CO_2 content [TCO_2]) are obtained on which subsequent decisions are based. The TCO_2, which is the sum of the [HCO_3^-] and dissolved CO_2 and usually is determined on a venous serum sample, must be distinguished from the PCO_2, which refers to the partial pressure of CO_2 that is generally measured in arterial blood. Once these laboratory studies are performed:

1. Examine the pH. Many students get confused by the potential complexity and forget this important and easy first step. Based on a normal sea-level pH of 7.42 ± 0.02, significant reduction in pH means that the major process ongoing is an acidosis. Conversely, a significant increase in pH means that the major process ongoing is an alkalosis.
2. Examine the directional changes of PCO_2 and [HCO_3^-] from normal. If the pH is acid and HCO_3^- is low, then metabolic acidosis must be present. Conversely, if the pH is alkalemic and HCO_3^- is high, then a metabolic alkalosis must be present.
3. Calculate the SAG. The SAG was discussed in detail earlier in this chapter. This useful calculation helps both in the differential diagnosis of metabolic acidosis and in identifying whether metabolic acidosis and alkalosis processes coexist (see below).
4. Calculate the Δ gap ($\Delta SAG - \Delta HCO_3^-$). In the setting of increased SAG metabolic acidosis, calculating the Δ gap can be very helpful in detecting a coexisting metabolic alkalosis or hyperchloremic metabolic acidosis. If the Δ gap is significantly positive (>6), this suggests that there is a coexisting metabolic alkalosis. Conversely, if Δ gap is significantly negative (<−6), a coexisting hyperchloremic metabolic acidosis is usually present.
5. Assess the degree of compensation. Is this a simple (compensation appropriate) or mixed acid–base disorder? With metabolic acidosis, the

PCO_2 (in torr) should decrease; conversely, with metabolic alkalosis, the PCO_2 should increase (Table 3-6). The rules of thumb for adequate compensation are presented in Table 3-7 and displayed graphically in Figure 3-6. Failure of respiratory compensation is equivalent to the presence of a primary respiratory acid–base disturbance. Note that a normal pH is never obtained for a given patient through compensation alone.

6. Determine the underlying cause of the disturbance. Acid–base disorders are merely laboratory signs of an underlying disease of the body fluids. The pathologic cause often is obvious once one has determined what the pathophysiologic nature of the acid–base disturbance is.
7. Determine appropriate therapy. In some situations, the acid–base disturbance must be directly addressed; however, in all situations, treatment of the underlying causes is most advantageous.

Table 3–6 Simple Acid–Base Disorders

Type of Disorder	pH	$PaCO_2$	$[HCO_3^-]$
Metabolic acidosis	↓	↓[a]	↓
Metabolic alkalosis	↑	↑[a]	↑
Respiratory acidosis	↓	↑	↑[a]
Respiratory alkalosis	↑	↓	↓[a]

[a]Change owing to compensation.

Table 3–7 Rules of Thumb for Bedside Interpretation of Acid–Base Disorders

Metabolic acidosis	$PaCO_2$ (in torr) should fall by 1−1.5× the fall in plasma $[HCO_3^-]$ (in mmol/L)
	$PaCO_2$ (in torr) should increase by 0.25−1× the rise in

	plasma [HCO_3] (in mmol/L)
Acute respiratory acidosis	The plasma [HCO_3] should rise by 0.1× the increase in $PaCO_2$ (in torr) ± 3 (in mmol/L)
Chronic respiratory acidosis	The plasma [HCO_3] should rise by 0.4× the increase in $PaCO_2$ (in torr) ± 4 (in mmol/L)
Acute respiratory alkalosis	The plasma [HCO_3] (in mmol/L) should fall by 0.1−0.3× the decrease in $PaCO_2$ (in torr) but usually not to <18 mmol/L
Chronic respiratory alkalosis	The plasma [HCO_3] (in mmol/L) should fall by 0.2−0.5× the decrease in $PaCO_2$ (in torr) but usually not to <14 mmol/L

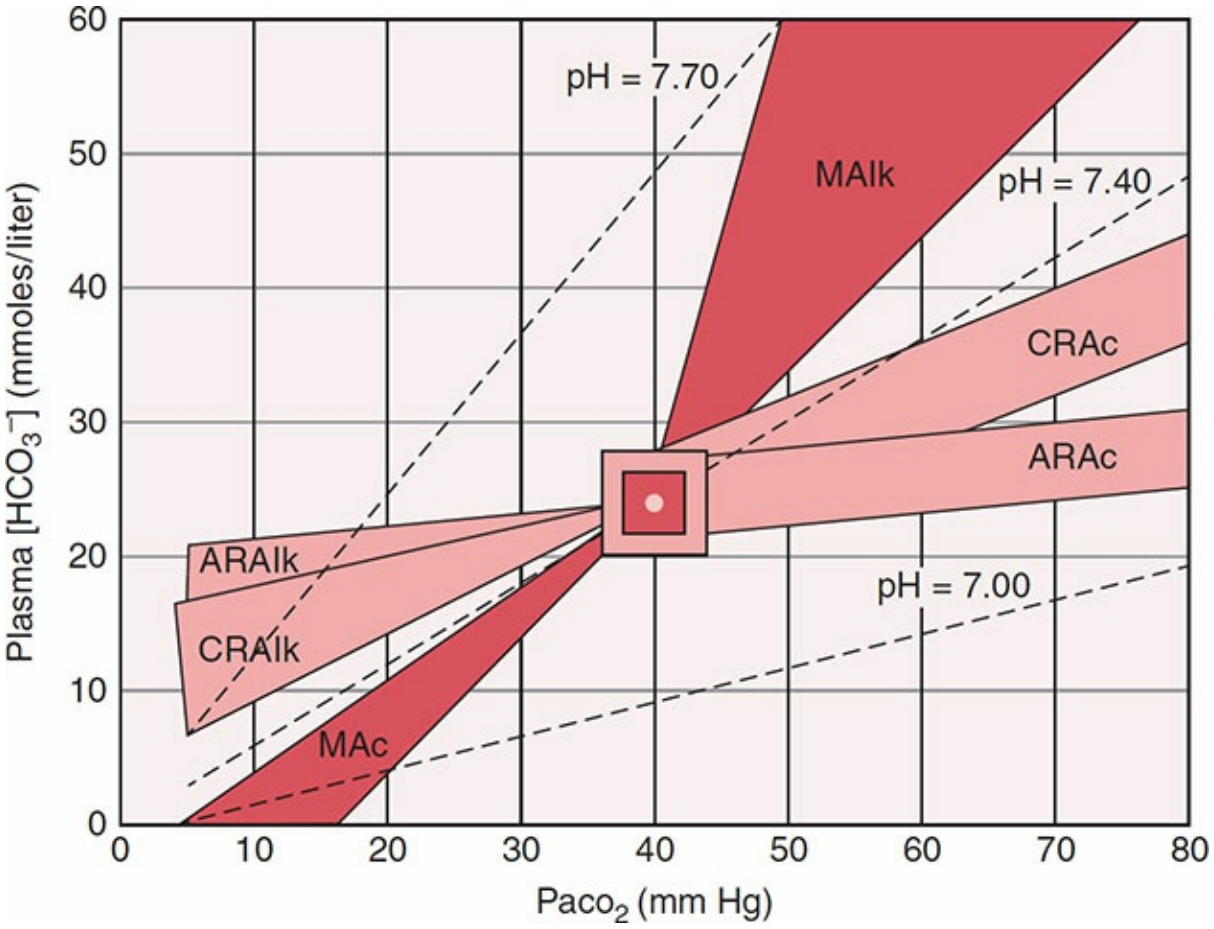

Figure 3–6 Acid–base nomogram derived from Table 3.7 is demonstrated. Regions on nomograms associated with plasma HCO_{3-} and $PaCO_2$ values seen with primary metabolic alkalosis (MAlk), metabolic acidosis (MAc), chronic respiratory acidosis (CRAc), chronic respiratory alkalosis (CRAlk), acute respiratory acidosis (ARAc), and acute respiratory alkalosis (ARAlk), and appropriate compensation are shown.

acute respiratory alkalosis (ARAlk), and appropriate compensation are shown.

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CHAPTER 4

Pathophysiology and Management of Respiratory and Mixed Acid–Base Disorders

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Respiratory acid–base disorders are caused by *primary* changes from normal excretion of carbon dioxide (CO_2) by the lungs. *Primary* means that the changes are not secondary to changes in pH caused by metabolic acid–base disorders. Under usual metabolic conditions, the body makes 13,000 to 15,000 mmol of CO_2 per day from the catabolism of carbohydrate, protein, and fat. If the lungs excrete this amount, the quantity of CO_2 in the body remains the same. This is reflected by the amount of CO_2 dissolved in blood and the partial pressure of the CO_2 gas in equilibrium with it (PCO_2). A small amount of the dissolved CO_2 reacts with water to form carbonic acid (H_2CO_3), the acid part of the Henderson–Hassel balch acid–base equation discussed in Chapter 3 and in detail elsewhere (1). This relationship between PCO_2 and pH is shown below:

$$pH \leftarrow \frac{[HCO_3^-]}{PaCO_2} \quad (4.1)$$

If the lungs' excretion does not match the daily production of CO_2, the quantity of CO_2 in the body changes; therefore, the amount dissolved in the blood and the pressure it generates (PCO_2) change in the same direction. This change generates either of the two simple (or primary) respiratory acid–base disorders: hypercapnia, or high CO_2 level, generates respiratory acidosis; hypocapnia, or low CO_2 level, generates respiratory alkalosis.

The whole body responds to changes in CO_2 content in a programmed fashion. In step 1, the pH change causes rapid chemical *buffering*. Buffers within cells either take up hydrogen ions [H^+], producing a bicarbonate [HCO_3^-] in the blood, or give up H^+ to titrate (consume) HCO_3^- in the blood. In step 2, the abnormal blood PCO_2 alters the renal tubular cell PCO_2, causing changes in H^+ secretion that result in changes in renal net acid excretion (NAE) that, in turn, raise or lower blood [HCO_3^-]. This process is called by its traditional name of *compensation*, but it is more of an *adaptation* to a new state of CO_2 balance. This process takes days to reach a new steady state. Thus, it occurs only in *chronic* respiratory acid–base disorders. In step 3, the respiratory system *corrects* the problem and restores the whole-body CO_2 content and the arterial PCO_2 ($PaCO_2$) to previously normal values. Obviously, this can occur only if the causative disorder is cured or corrected. Notably, changes in oxygen (O_2) supply and demand and the PaO_2 do not define respiratory acid–base disorders, but they can cause both respiratory acid–base disorders and metabolic acidosis through their effects on respiratory drive and lactic acid metabolism (2).

Carefully obtained arterial blood for analysis is the usual way of diagnosing respiratory acid–base disorders. There are a variety of approaches used to correctly classify acid–base disorders (3). A commonly used approach is a physiologic one based on the Henderson–Hasselbach equation. To accurately identify an acid–base disorder using this approach, one needs measurements of pH, PCO_2, HCO_3, and the anion gap. This approach uses only the carbonic acid/bicarbonate buffer system to assess acid–base disorders and is the approach that will be used in this chapter. The two other approaches to acid–base disorders are the base excess approach and the physicochemical approach (Stewart approach). The

former analysis takes into consideration all buffer systems in the body, whereas the latter approach measures the variables of strong ion difference and weak acid concentration. These two approaches have been reviewed extensively (3) and will not be discussed further in this chapter.

Respiratory Acidosis

Respiratory acidosis is a disorder caused by processes that increase P_{CO_2}, which thereby leads to a decrease in the pH. The P_{CO_2} increases when the lungs fail to excrete metabolically produced CO_2. A decrease in effective alveolar ventilation is the usual way that the P_{CO_2} is increased. Effective alveolar ventilation can be diminished in two major ways, namely, decreased minute ventilation or ventilation–perfusion inequality (4). If effective ventilation is fixed by a ventilator or respiratory disease, the introduction of nutrition, parenteral or enteral, will increase the generation of CO_2. Using glucose as the source of nonprotein calories increases CO_2 production by 20% compared with glucose–lipid mixtures (5).

With decreased effective alveolar ventilation, CO_2 excretion falls short of production, and the quantity of CO_2 carried per milliliter of blood increases, as reflected in an increased Pa_{CO_2}. When a steady state of hypercapnia is reached, the ventilatory excretion of CO_2 again equals production. This new state occurs because a higher concentration of CO_2 is carried to the pulmonary vascular bed, allowing more CO_2 excretion.

When the Pa_{CO_2} rises, the amount of dissolved CO_2 increases and shifts the equilibrium reaction to favor the production of H_2CO_3; thus, $CO_2 + H_2O \rightarrow H_2CO_3$. This increased acid results in a fall in pH or respiratory acidosis. This process can be visualized more simply as a rise in Pa_{CO_2}, which reduces the ratio of the HCO_3^- concentration to the Pa_{CO_2}, thereby causing a fall in pH:

$$\downarrow \text{pH} \leftarrow \frac{[HCO_3^-]}{\uparrow Pa_{CO_2}} \qquad (4.2)$$

The chemistry of these reactions is discussed in Chapter 3.

PATHOPHYSIOLOGY OF RESPIRATORY ACIDOSIS

Buffering

The immediate response to the low pH generated by the increased $PaCO_2$ and H_2CO_3 is to buffer (or bind) hydrogen ions with nonbicarbonate buffers. Bicarbonate does not work as an effective buffer in this situation because it reacts with hydrogen ions to form H_2CO_3, which is the original culprit. In the extracellular fluid (ECF) space, proteins constitute the only buffer, whereas within the cells, hemoglobin, phosphate, proteins, and lactate are the major nonbicarbonate buffers; 97% of the buffering of H_2CO_3 derives from intracellular rather than extracellular fluid buffers (6).

Renal Compensation

The definitive compensation for respiratory acidosis resides solely in the kidneys. The kidneys respond to the increased systemic PCO_2 by increasing the production and excretion of ammonium (NH_4^+). The renal tubular cells metabolize glutamine to produce two NH_3 molecules and α-ketoglutarate (AKG). The AKG goes to the liver, where metabolism produces two HCO_3^-. The kidney excretes the NH_3 as NH_4^+, which marks the addition of the new HCO_3^- to the body stores by the liver. If the kidneys fail to excrete the NH_4^+, it travels to the liver, and metabolism generates H^+, which negates the addition of the HCO_3^- to the body. Thus, increased renal excretion of NH_4^+ is a crucial component of the generation of new HCO_3^-. The increased urinary NH_4^+ excretion is balanced by increased Cl^- excretion, with a resultant fall in plasma (Cl^-). When a steady state of hypercapnia is reached, chloride excretion returns to normal and equals intake. NH_4^+ excretion also returns to normal, even though H^+ secretion remains increased. The persistently increased H^+ secretion is needed to reclaim the increased filtered HCO_3^- load that results from the increase in plasma concentration (7,8). Experimental evidence has shown that both the proximal tubule and the distal tubule participate in adaptation to respiratory acidosis. In the proximal tubule, increases in $PaCO_2$ cause corresponding activation of the luminal Na/H exchanger and basolateral Na/HCO_3 exchanger, leading to net reabsorbtion of bicarbonate (9). There

also appears to be increased activity of H^+/ATPase in the distal tubule (10). The chemical buffering and renal compensation that occur with chronic respiratory acidosis are diagramed in Figure 4-1.

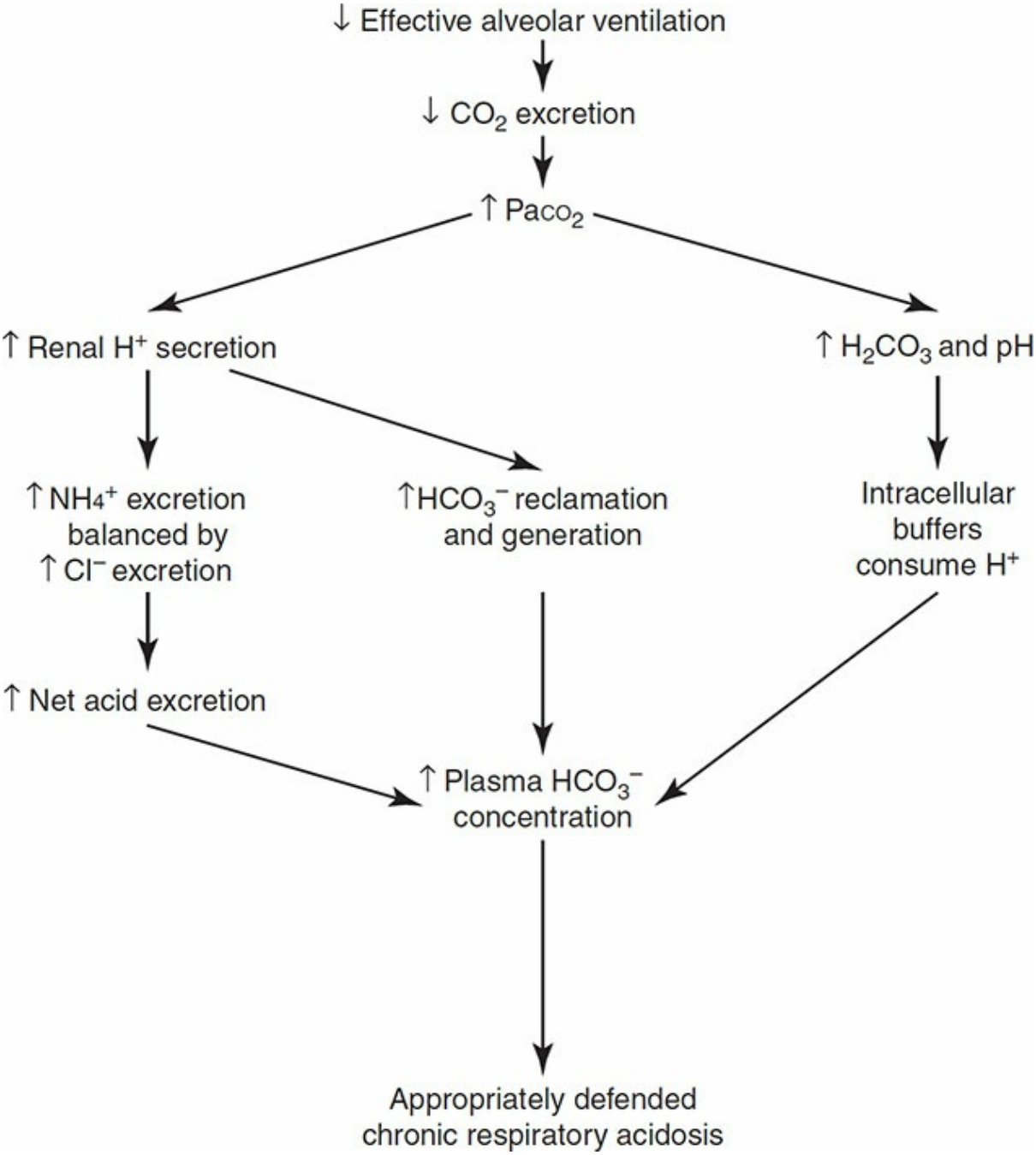

Figure 4–1 Pathophysiology of chronic respiratory acidosis. Chemical buffering and renal compensation combine to elevate the plasma [HCO_{3-}]. The renal mechanisms involve an adaptive increase in ammonium and chloride excretion until the new steady state is reached.

Correction of Respiratory Acidosis

The third, or corrective, response to respiratory acidosis is the restoration of effective ventilation. Correction or amelioration of an acute neurologic process causing hypoventilation or a ventilatory or gas-exchange defect may be possible. Unfortunately, many processes that result in chronic hypercapnia are caused by irreversible parenchymal lung damage and thus can be corrected only partially at best.

In metabolic acidosis, the corrective agent, the kidney, is sometimes a cause of the disorder, as in uremic acidosis, but at other times is not, as in diabetic ketoacidosis. In respiratory acidosis, however, the respiratory system, which includes neural control—mechanical, circulatory, and membrane exchange components—always is involved as the cause of the disorder and is also the corrective agent.

ACUTE RESPIRATORY ACIDOSIS (ACUTE HYPERCAPNIA)

Acute respiratory acidosis results from acute alveolar hypoventilation with a primary elevation of the $PaCO_2$, when only the buffering defense has had time to come into play. Although buffering occurs almost immediately, the renal response does not exert a noticeable influence for 12 to 24 hours (11). This is the period of time in which pure acute respiratory acidosis is observed. An appropriate defense of pH in acute respiratory acidosis is characterized by an elevation of the plasma $[HCO_3^-]$ by about 1 mmol/L ($^>$24) for each 10 mm Hg acute increment in $PaCO_2$ ($^>$40) (12):

$$\Delta[HCO_3^-] = \left(\frac{\Delta PaCO_2}{10}\right) \pm 3 \qquad (4.3)$$

Clinical Features and Systemic Effects of Acute Respiratory Acidosis

The acute onset of hypercapnia is invariably accompanied by hypoxemia, which usually dominates the clinical picture. Depending on the underlying disorder and the state of consciousness, the patient may present with the signs and symptoms of acute respiratory distress, including marked restlessness, tachypnea, and marked dyspnea. As the process worsens, stupor and eventually coma develop. CO_2 has vasodilating properties, and thus hypercapnia is associated with increased cerebral blood flow (13,14). This increase in blood flow to the brain probably accounts for the headaches and occasional signs of increased intracranial pressure that can

occur with both acute and chronic hypercapnia (15,16). Severe acute respiratory acidosis can cause refractory hypotension through two mechanisms (17). First, cardiac contractility is reduced and cardiac output falls. Second, peripheral arterial smooth muscle relaxes, causing vasodilation and decreased systemic vascular resistance. Modest acute increases in $PaCO_2$ (13–19 mm Hg) actually increase cardiac output as well as pulmonary artery pressure (18).

In intensive care units, hypoventilation is frequently induced in patients with severe lung injury and acute respiratory distress syndrome (ARDS). This strategy was shown to reduce mortality and ventilator time in the ARDSNet trial (19). Although the difference in $PaCO_2$ was not large (5 mm Hg), this trial did suggest that the benefits of low tidal volume ventilation outweigh the risks of mild acute hypercapnia. It should be noted that many patients in the trial were treated with bicarbonate therapy to minimize the fall in blood pH.

Laboratory Findings in Acute Respiratory Acidosis

With acute respiratory acidosis, the arterial blood reflects the pathophysiologic state with an elevated PCO_2, a moderately elevated plasma [HCO_3^-] ($<$30 mmol/L), and a low pH. If the patient is breathing room air, then the PaO_2 is decreased. The venous serum electrolytes reveal a modestly elevated total CO_2 content, with usually normal plasma concentrations of sodium, potassium, and chloride.

Causes of Acute Respiratory Acidosis

Some of the causes of acute respiratory failure, which leads to acute CO_2 retention, are listed in Table 4-1.

Treatment of Acute Respiratory Acidosis

The key to treatment of acute respiratory acidosis is the restoration of effective ventilation. Modest amounts of sodium bicarbonate ($NaHCO_3$) may be given intravenously to mitigate severe acidosis; the latter is only a holding measure to prevent the serious cardiovascular effects of marked acidemia until definitive therapy is established (20). Because equilibration of HCO_3^- across the blood–brain barrier is markedly slower than that of

CO_2 with bicarbonate administration, a delay in the correction of the cerebral pH may occur, and cerebrospinal fluid pH falls initially (21).

Table 4–1 Causes of Acute Respiratory Acidosis

Neuromuscular Abnormalities
Brainstem injury
High cord injury
Guillain–Barré syndrome
Myasthenia gravis
Botulism
Narcotic, sedative, or tranquilizer overdose
Status epilepticus
Postoperative hyponatremia with herniation
Airway Obstruction
Foreign body
Aspiration of vomitus
Laryngeal edema
Severe bronchospasm
Thoracic–Pulmonary Disorders
Flail chest
Pneumothorax
Severe pneumonia
Smoke inhalation
Severe pulmonary edema
Vascular Disease
Massive pulmonary embolism
Respirator-Controlled Ventilation
Low rate and/or tidal volume
Large dead space
Total parenteral nutrition (increased CO_2 production)

CHRONIC RESPIRATORY ACIDOSIS (CHRONIC HYPERCAPNIA)

Chronic respiratory acidosis is caused by chronic decreased effective alveolar ventilation with a primary elevation of the $PaCO_2$. The duration of the elevation of $PaCO_2$ must be sufficient to permit adaptation of the renal mechanisms to be maximized. In dogs, a new steady state of blood acid–base values occurs 5 days after the onset of hypercapnia (11). The exact time interval needed to establish "chronic" hypercapnia in humans has not been established. A quantitative relationship has been described between the steady-state $PaCO_2$ and the H^+ concentration in patients with chronic hypercapnia. This relationship is linear and is described by a slope of about 0.25 nmol of H^+ per 1 mm Hg increase in $PaCO_2$ (22,23). A clinical guide for bedside use expresses the relationship between $PaCO_2$ and plasma $[HCO_3^-]$ in chronic hypercapnia as follows: For each increment of 10 mm Hg in $PaCO_2$, the plasma $[HCO_3^-]$ rises by 4 mmol/L, with a range of 4 mmol/L in either direction. The following formula summarizes this rule of thumb:

$$\Delta \text{Plasma}\left[HCO_3^-\right] = 4 \times \left(\frac{\Delta PaCO_2}{10}\right) \pm 4 \text{ mmol / L} \qquad (4.4)$$

Clinical Features and Systemic Effects of Chronic Respiratory Acidosis

Patients with chronic respiratory acidosis exhibit few, if any, signs or symptoms related directly to CO_2 retention and acidosis. However, papilledema and other neurologic disturbances have been described in several patients (22,23). These findings are not caused by the hypercapnia per se nor by the pH changes that mediate cerebral vascular reactivity through intracellular calcium. Rather, secondary changes in catecholamines are the likely causes (13). The signs and symptoms of the chronic pulmonary disease, with or without cor pulmonale, usually predominate. Chronic respiratory acidosis causes decreased bone mineralization, although to a lesser degree than does metabolic acidosis (24–26). This effect does not appear to be mediated by altered function of bone osteoclasts or osteoblasts and is not accompanied by hypercalciuria (27,28).

Laboratory Findings in Chronic Respiratory Acidosis

Arterial blood examination reveals a low pH (not $^{<}$7.25 even with severe chronic CO_2 retention), an elevated $PaCO_2$, and an elevated plasma [HCO_3^-]. Thus, a blood pH $^{<}$7.25 is a marker of imposed acute hypercapnia or metabolic acidosis. Plasma sodium and potassium concentrations are usually normal. Total plasma CO_2 content is elevated, and plasma chloride concentration is reciprocally decreased. The anion gap is usually normal (22). In the absence of diuretic use or vomiting, these serum electrolyte findings should lead the clinician to check arterial blood gas values. The urine pH is usually acid.

Causes of Chronic Respiratory Acidosis

Chronic respiratory acidosis is seen most commonly in patients with chronic obstructive pulmonary disease (COPD). However, any condition that can lead to chronic retention of CO_2 (27) will cause the same acid–base disturbance. Examples of such conditions are given in Table 4-2.

The epidemic of obesity has led to an increased prevalence of the obesity hypoventilation syndrome (OHS). Patients with OHS exhibit hypercapnia while awake ($PaCO_2$ $^{>}$ 45 mm Hg), obesity (BMI $^{>}$ 30 kg/m^2), and absence of alternative causes of hypoventilation. Most patients with OHS have sleep-disordered breathing and obstructive sleep apnea. The hypoventilation may be secondary to altered respiratory muscle mechanics and decreased ventilator drive (29,30). Patients with OHS commonly develop hypoxemia, pulmonary hypertension, and signs of right-sided congestive heart failure. In patients with nocturnal hypercapnia due to sleep apnea but daytime normocapnia, metabolic alkalosis may actually develop. Nocturnal hypercapnia can lead to renal generation of bicarbonate. People on low-salt diets (low chloride) are unable to excrete this bicarbonate and thus develop posthypercapnic metabolic alkalosis.

Table 4–2 Causes of Chronic Respiratory Acidosis

Neuromuscular Abnormalities
Chronic narcotic or sedative ingestion
Primary hypoventilation
Pickwickian syndrome
Poliomyelitis
Diaphragmatic paralysis
Hypothyroidism

Sleep apnea syndrome
Thoracic–Pulmonary Disorders
Chronic obstructive pulmonary disease Kyphoscoliosis End-stage interstitial pulmonary disease

Treatment of Chronic Respiratory Disease

Chronic respiratory acidosis can be corrected effectively only by restoring or improving the ability of the respiratory system to excrete CO_2. Often, this is impossible because of an irreversible pathologic condition. However, adequate airway drainage, relief of bronchospasm, and treatment of pulmonary infections and congestive heart failure may lead to significant improvement. Because the arterial pH remains $^{>}$7.25 even with chronic $PaCO_2$ elevations to 110 mm Hg (31), the acidosis per se is not dangerous, although the patient is at more risk for serious acidemia if metabolic acidosis occurs. Attention to the maintenance of adequate O_2 tension (PO_2) is the critical need.

People with end-stage renal disease (ESRD) who also have COPD or another cause of chronic hypercapnia are unable to generate the usual amounts of bicarbonate for compensation in chronic respiratory acidosis. Thus, they may benefit from dialysis using a higher bicarbonate dialysate.

ACUTE HYPERCAPNIA SUPERIMPOSED ON CHRONIC RESPIRATORY ACIDOSIS

When a patient in a steady state of chronic hypercapnia suffers a new insult to his or her ability to excrete CO_2, the $PaCO_2$ rises acutely to a new level. Thus, the plasma [HCO_3^-] and blood pH are lower than predicted for a given chronic level of $PaCO_2$. However, the change in pH is not as great as would be expected for a similar acute increment in $PaCO_2$ occurring in a previously normal person. That is, the pH is better protected against an acute rise in $PaCO_2$ when there is a background of chronic respiratory acidosis than it is with acute respiratory acidosis alone (32,33). The mechanism for this is not entirely clear but has been attributed partially to the physicochemical effect of the preexisting higher [HCO_3^-], which would reduce the fall in pH compared with that seen for a similar

increment in $PaCO_2$ in a patient with a normal $[HCO_3^-]$. In addition, the kidney rapidly increases H^+ excretion when an acute rise in PCO_2 is superimposed on chronic respiratory acidosis. This is in contrast to acute respiratory acidosis alone, in which renal acid excretion makes little quantitative contribution to H^+ balance. Treatment is directed toward correcting the acute disorder and providing supplemental O_2.

Respiratory Alkalosis

Respiratory alkalosis is caused by a process that leads to a rise in pH owing to a primary decrease in the $PaCO_2$. The $PaCO_2$ can fall only if the excretion of CO_2 by the lungs exceeds the production of CO_2 by metabolic processes. Because the production of CO_2 usually remains relatively constant, a negative CO_2 balance results primarily from increased alveolar ventilation. Hyperventilation can result from two processes: (a) increased neurochemical stimulation of ventilation by central or peripheral neural mechanisms and (b) physically increased ventilation, either artificially with mechanical ventilators or voluntarily with increased conscious effort. Alveolar hyperventilation produces increased CO_2 excretion, which reduces the $PaCO_2$ and H_2CO_3. This fall in $PaCO_2$ increases the ratio of the $[HCO_3^-]$ to $PaCO_2$, which results in a rise in the pH of the blood, that is, alkalemia.

$$\uparrow \text{pH} \leftarrow \frac{[HCO_3^-]}{\downarrow PaCO_2} \qquad (4.5)$$

The buffering response constitutes the *acute* phase of respiratory alkalosis, whereas the renal response to hypocapnia defines the *chronic* stage of respiratory alkalosis.

PATHOPHYSIOLOGY OF RESPIRATORY ALKALOSIS

Buffering

Buffering constitutes the first response in respiratory alkalosis. To return the pH toward normal in the face of the decreased H_2CO_3 or $PaCO_2$, the

plasma [HCO_3^-] must be decreased. Therefore, H^+ is released from the body buffers, and the plasma [HCO_3^-] is reduced by the following net reaction:

$$H^+ + HCO_3^- \rightarrow CO_2 + H_2O \quad (4.6)$$

Intracellular buffers supply about 99% of the H^+, whereas plasma proteins contribute about 1% to the buffering effort (6). Cellular metabolism contributes by increasing the production of lactic acid and possibly of slight amounts of other organic acids. Lactate concentration increased by 0.5 mmol/L in a study in anesthetized patients; this represents about 10% of the total buffering effort. Buffering is completed within minutes, and the steady state persists for at least 2 hours (34). The alacrity of the response is critical because the $PaCO_2$ can decrease abruptly, and without buffering, life-threatening alkalemia would occur. The quantitative change in plasma [HCO_3^-] is not great, however, and the pH therefore may rise markedly. The arterial [HCO_3^-] fell to as low as 18 mmol/L at $PaCO_2$ levels of 15 to 20 mm Hg in anesthetized patients (35). A rule of thumb for acute respiratory alkalosis is that the [HCO_3^-] should decrease by 1 mmol/L for each 10 mm Hg decrement in $PaCO_2$:

$$\Delta[HCO_3^-] = 1 \times \left(\frac{\Delta PaCO_2}{10}\right) \pm 3 \quad (4.7)$$

Renal Compensation

The second adaptive response in respiratory alkalosis is handled by a renal mechanism. The kidney attempts to lower the plasma [HCO_3^-] in either of two ways: by decreasing the reclamation of filtered HCO_3^-, thus leading to bicarbonaturia, or by reducing the generation of new HCO_3^- to replace that consumed in the daily buffering of the dietary metabolic acid load. In animals, the kidney appears to make the second choice because a decreased NH_4^+ excretion without an increased HCO_3^- excretion occurs during the phase of adaptation to chronic hypocapnia (35). This reduction in excretion of NH_4^+, a cation, is balanced electrochemically by increased sodium or potassium excretion (36). After a new steady state is reached, excretion of these electrolytes returns to normal. The process of renal

adaptation appears to occur rapidly and is probably completed within 24 to 48 hours (36). In humans, the early stage of renal adaptation for prolonged hypocapnia is characterized by bicarbonaturia, natriuresis, and decreased NH_4^+ and titratable acid excretion (37,38). The stimulus for this renal response appears to be independent of systemic pH changes but is a direct effect of the $PaCO_2$ level on renal reabsorption of an anion, either HCO_3^- or chloride (39,40). The chemical buffering and proposed renal response in respiratory alkalosis are diagramed in Figure 4-2.

The quantitative contribution of the kidney to pH defense is difficult to judge in humans. Subjects with hypocapnia caused by voluntary hyperventilation or altitude hypoxemia had decreases in plasma $[HCO_3^-]$ of 2.1 to 4.9 mmol/L/10 mm Hg decrease in $PaCO_2$ during 1 to 11 days of hypocapnia (37,38,41). Arterial pH remained frankly alkalemic. Climbers adapted to altitude over 60 days had $PaCO_2$ levels of 13.3 and $[HCO_3^-]$ of 10.8 mmol/L with resultant pH of 7.53 just below the Everest summit (42). However, some studies of lifelong high-altitude dwellers have shown decreased plasma $[HCO_3^-]$ sufficient to produce pH values of 7.4 with $PaCO_2$ levels as low as 28 to 30 mm Hg (43–46). For clinical purposes, a useful rule is to diagnose simple chronic respiratory alkalosis when plasma $[HCO_3^-]$ is decreased 4 mmol/L $^{<}24$ for each 10 mm Hg chronic decrement $^{<}40$ in the $PaCO_2$.

$$\Delta\left[HCO_3^-\right] = 4 \times \left(\frac{\Delta PaCO_2}{10}\right) \pm 2 \qquad (4.8)$$

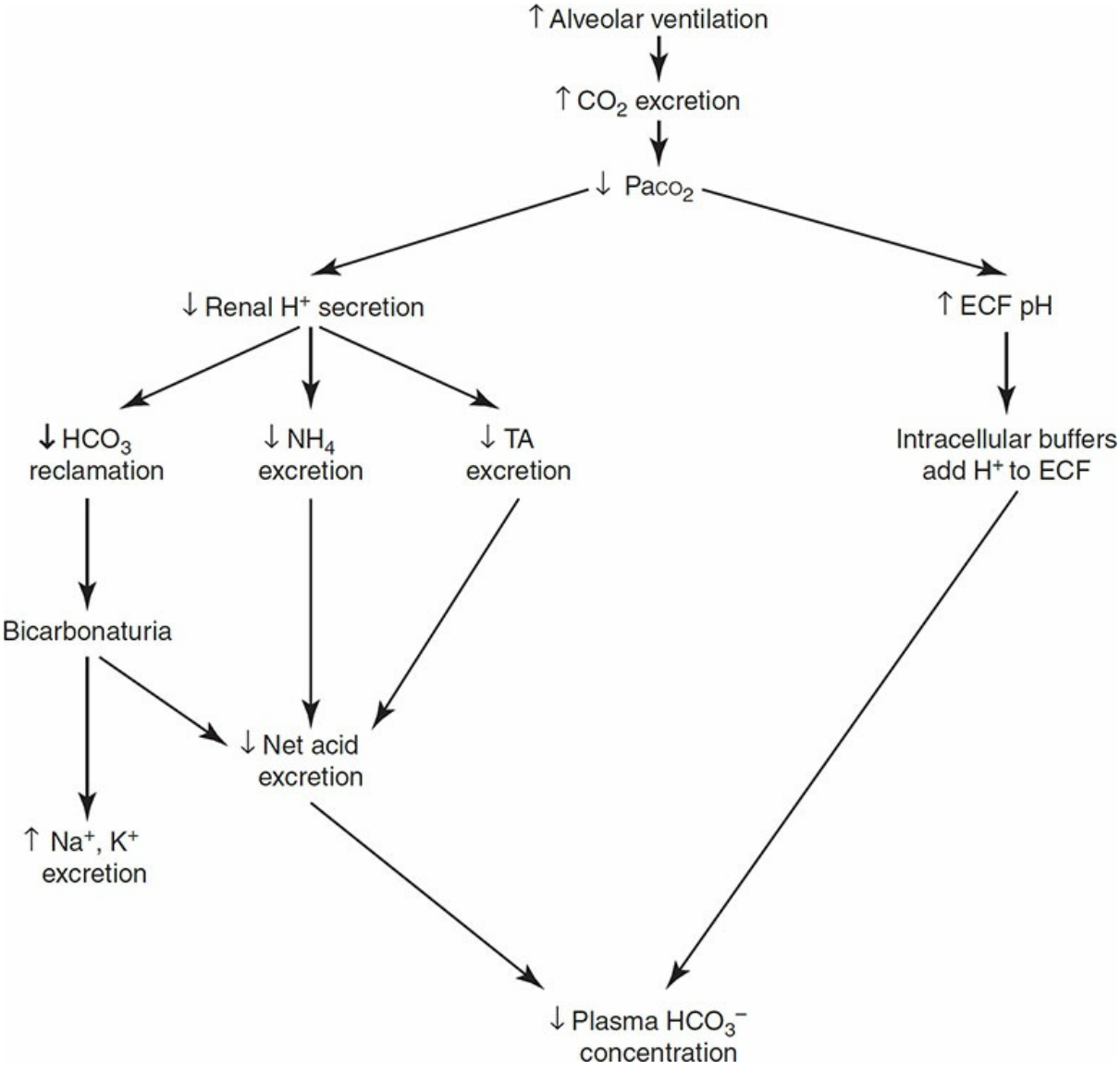

Figure 4–2 Pathophysiology of chronic respiratory alkalosis. Intracellular buffers donate H^+ to the ECF to produce a small decrease in plasma [HCO_{3-}] during acute hypocapnia. The net effect on renal function of prolonged hypocapnia is a decrease in H^+ secretion, which results in a fall in net acid excretion below the level necessary to maintain acid balance. Thus, plasma [HCO_{3-}] level falls. Urinary acid and electrolyte excretion return to normal after steady-state $PaCO_2$ and [HCO_{3-}] are achieved. ECF, extracellular fluid; TA, titratable acid.

Correction of Respiratory Alkalosis

The third, or corrective, response in respiratory alkalosis entails correction of the hyperventilation that maintains the negative CO_2 balance. This, of course, is dependent on removal of the neurohumoral stimulus to the respiratory center or cessation of mechanical or voluntary hyperventilation. The latter is easier to achieve because neural stimulation

of ventilation is often caused by pathophysiologic processes that are difficult to correct.

CLINICAL FEATURES AND SYSTEMIC EFFECTS OF RESPIRATORY ALKALOSIS

Hypocapnia may be manifested by symptoms and signs of neuromuscular irritability. Patients may complain of perioral and extremity paresthesias, muscle cramps, and even tinnitus. Hyperreflexia, tetany, and even seizures may occur (47,48). Hypocapnia causes cerebral vasoconstriction with reduced blood flow, which may have deleterious and even fatal effects on the brain, especially in patients with sickle cell disease (49–52). Marked alkalemia may cause serious refractory cardiac arrhythmias and electrocardiographic changes of ischemia (53–57).

LABORATORY FINDINGS WITH RESPIRATORY ALKALOSIS

If arterial blood pH is increased, then a decreased $PaCO_2$ and plasma $[HCO_3^-]$ are diagnostic of respiratory alkalosis. Venous serum total CO_2 content reflects the decrease in $[HCO_3^-]$, and chloride concentration is slightly increased. Serum potassium is increased by an average of 0.3 mmol/L in acute respiratory alkalosis. This effect appears to be stimulated by the buffering-induced fall in $[HCO_3^-]$, which activates the α-adrenergic system (Chapter 5) (58). Serum phosphorus concentration may decrease only slightly or to seriously low levels (Chapter 6) (59–61). Urine pH is not clinically informative. It may be relatively alkaline ($^{>}$6.0) during the onset of acute hypocapnia but then fluctuates into the more acidic range, as in the eucapnic state (37).

DIFFERENTIAL DIAGNOSIS OF RESPIRATORY ALKALOSIS

Respiratory alkalosis is the most common acid–base disorder among seriously ill patients (62). The reason for this is apparent from a review of the list of causes of respiratory alkalosis (Table 4-3).

Central stimulation of the medullary respiratory center occurs with anxiety, pain, pregnancy, febrile states, and salicylate intoxication. Mechanical irritation by brain trauma or tumor is another respiratory stimulant.

Stimulation of the peripheral pathways to the medullary respiratory center occurs in pulmonary–thoracic disorders that cause hypoxemia with relatively unimpaired CO_2 transport, altitude hypoxemia, asthma (63), and disorders that decrease lung compliance (stiff lung) without necessarily causing hypoxemia.

Mechanical ventilation can be associated with respiratory alkalosis if the rate and/or tidal volume are inappropriately high. While most forms of respiratory alkalosis are associated with mild alkalemia, this situation can often be associated with severe alkalemia (pH $>$ 7.55) (64).

Patients with hepatic cirrhosis often have respiratory alkalosis (65). The mechanism is probably multifactorial (66), although increased pulmonary shunting (67), hyponatremia (68), and increased blood ammonia levels (69) have been implicated. Respiratory alkalosis is an early manifestation of gram-negative sepsis and other forms of shock; therefore, the clinician should suspect these processes in the appropriate clinical setting (70).

TREATMENT OF RESPIRATORY ALKALOSIS

The only definitive treatment is to correct or ameliorate the basic disorder responsible for the hyperventilation. Correcting significant hypoxemia is more critical for the patient's well-being than is correcting the acid–base disturbance. If alkalemia is causing deleterious neuromuscular or cardiac rhythm problems in a patient on mechanical ventilation, then decreasing the minute ventilation or increasing the dead space may be effective. If this cannot be done without compromising oxygenation, then the use of an inhaled gas mixture containing 3% CO_2 may be helpful for short periods of time (71).

Table 4–3 Causes of Respiratory Alkalosis

Central Stimulation of Respiration
Anxiety
Head trauma
Brain tumors or vascular accidents
Salicylates
Fever
Pain
Pregnancy

Peripheral Stimulation of Respiration
Pulmonary emboli Congestive heart failure Interstitial lung diseases Pneumonia “Stiff lungs” without hypoxemia Altitude Asthma
Multiple Mechanisms
Hepatic insufficiency Gram-negative septicemia
Mechanical or Voluntary Hyperventilation

Mixed Acid–Base Disorders

Mixed acid–base disorders occur when two or even three primary events act independently to alter the acid–base state at the same time. Five double- and two triple-mixed acid–base disorders can occur as listed in Table 4-4. The two primary respiratory acid–base disorders, respiratory acidosis and respiratory alkalosis, cannot coexist.

DIAGNOSIS OF MIXED ACID–BASE DISORDERS

A mixed acid–base disorder can be suspected from the clinical setting (e.g., a patient with cor pulmonale on diuretics) and can be diagnosed from arterial blood and venous serum or plasma studies. The key to the diagnosis of a mixed disturbance is the clear understanding of the expected compensation in the primary, uncomplicated acid–base disorders. If the compensation is appropriate, the disorder is simple; if it is out of the expected range for an uncomplicated, primary disorder, a mixed disorder is suspected. To determine whether compensation is appropriate for a given disorder, it is essential to know the expected response. In Table 3-6 are shown the expected directional changes in pH, $PaCO_2$, and plasma [HCO_3^-] for each simple disorder. Thumb rules for estimating the expected changes in these values are listed in Table 3-7. A mixed disorder should be suspected if a given set of blood acid–base values does not fall within the range of the expected response for that acid–base disorder (72).

This is easy to judge if the bicarbonate and PCO_2 deviate from normal in opposite directions.

Certain of the mixed acid–base disorders may cause dangerous deviations of pH from normal, whereas others may produce pH values within the normal range. The dangerous combinations are those in which the primary disorders block the compensation for each other. For example, the hypercapnia of respiratory acidosis prevents the adaptive hypocapnia of metabolic acidosis, and the hypobicarbonatemia of metabolic acidosis blocks the adaptive rise in plasma [HCO_3^-] expected in respiratory acidosis. The dangerous disorders are thus characterized by *failure of compensation* (Table 4-4).

The "benign" mixed acid–base disorders are those in which the primary disorders provide *excessive compensation* for each other (Table 4-4). For example, salicylate intoxication may produce acidosis, such as plasma [HCO_3^-] of 10 mmol/L or a reduction of 14 mmol/L below normal sea-level values. Application of the rule of thumb for metabolic acidosis (Table 3-2) predicts the maximum fall in $PaCO_2$ to be 1.5 × 14 = 21 mm Hg. Thus, a $PaCO_2$ of $<$19 mm Hg (40 − 21) would not be appropriate for simple metabolic acidosis. Salicylate has a primary stimulating effect on ventilation, however, and may produce sufficient hyperventilation to lower the $PaCO_2$ to 14 mm Hg in this example. Thus, the primary hypocapnia would result in excessive compensation for the fall in [HCO_3^-] and pH produced by salicylate intoxication. Reciprocally, the fall in [HCO_3^-] would be greater than that predicted as appropriate for respiratory alkalosis (Table 3-2).

Table 4–4 Mixed Acid–Base Disorders

Disorders	Adaptation	pH
Inadequate Response		
Metabolic acidosis and respiratory acidosis	$PaCO_2$ too high and [HCO_3^-] too low for simple disorders	↓ ↓
Metabolic alkalosis and respiratory alkalosis	$PaCO_2$ too low and [HCO_3^-] too high for simple disorders	↑ ↑
Excessive Response		

Metabolic acidosis and respiratory alkalosis	$PaCO_2$ too low and $[HCO_3^-]$ too low for simple disorders	Normal or slightly ↓ or ↑
Metabolic alkalosis and respiratory acidosis	$PaCO_2$ too high and $[HCO_3^-]$ too high for simple disorders	Normal or slightly ↑ or ↓
Triple Disorders		
Metabolic alkalosis, metabolic acidosis, and either respiratory acidosis or alkalosis	$PaCO_2$ and $[HCO_3^-]$ not appropriate for simple disorders and anion gap >17 mEq/L	Variable

A nomogram for interpreting acid–base variables is displayed in Figure 3-6. A point falling outside the indicated predictive bands suggests the presence of a mixed acid–base disorder. However, a mixed disorder also may result in a set of acid–base variables falling within a band, as discussed in the figure legend; therefore, acid–base variables must be interpreted in light of the entire clinical circumstances and not as an isolated set of numbers.

COMMON MIXED ACID–BASE DISORDERS

Respiratory Acidosis and Metabolic Alkalosis

Patients with chronic lung diseases that produce CO_2 retention and respiratory acidosis often develop congestive heart failure. If diuretics are used to treat the heart failure, then the plasma $[HCO_3^-]$ may rise to levels greater than those appropriate for renal compensation in chronic respiratory acidosis (Table 3-2; Fig. 3-3). The pH may rise to the normal range or even frankly elevated levels. These changes may finally result in a set of acid–base variables appropriate for simple metabolic alkalosis, for example, plasma $[HCO_3^-]$, 48 mmol; $PaCO_2$, 60 mm Hg; and pH, 7.52. Clinical information, however, indicated that this particular set of laboratory values resulted from the coexistence of two primary acid–base disorders, chronic respiratory acidosis, with a primary increase in $PaCO_2$ and secondary compensatory rise in plasma $[HCO_3^-]$, and metabolic alkalosis, with a primary increase in $[HCO_3^-]$ above the expected level for

chronic respiratory acidosis.

Difficulty in interpreting the acid–base variables may arise if clinical information is not available or not clear as to the existence of lung disease with chronic CO_2 retention. In that instance, it is helpful to observe the patient's response to the cessation of diuretics and administration of sodium and potassium chloride. This treatment will correct simple metabolic alkalosis (Chapter 3) but achieve only some improvement in the $PaCO_2$ in the mixed disorder. A large alveolar–arterial PO_2 gradient ($^{>}$15 mm Hg) indicates lung disease and is suggestive of some component of respiratory acidosis.

Although this mixed disorder is one of the excessive compensation variety in which pH does not deviate markedly from normal, it should be treated to maintain the PaO_2 at the best attainable level. The increase in plasma [HCO_3^-] and concomitant rise in pH owing to diuretic-induced metabolic alkalosis are sufficient to suppress ventilation in patients with chronic respiratory acidosis, thus causing a decrease in PaO_2 (73). Treatment of this mixed disorder should be directed at lowering the plasma [HCO_3^-] through sodium chloride and potassium chloride therapy, which best allows the kidney to excrete HCO_3^- retained as a result of diuretic-induced metabolic alkalosis (Chapter 3). Of course, this therapy must be used with caution to avoid exacerbating volume overload. Although the pH will fall to acidemic levels, this is beneficial inasmuch as it stimulates ventilation, thus increasing PaO_2 and decreasing $PaCO_2$. In any event, pH in chronic respiratory acidosis is well defended and does not fall below 7.25, as discussed (30).

Respiratory Acidosis and Metabolic Acidosis

Mixed respiratory and metabolic acidosis may develop in patients with cardiorespiratory arrest, chronic lung disease who are in shock, and metabolic acidosis of any type who develop respiratory failure. This mixed disorder is a failure of compensation (Table 4-4). The respiratory disorder prevents the fall in $PaCO_2$ expected in the defense against metabolic acidosis. The metabolic disorder prevents the buffering and renal mechanisms from raising the plasma [HCO_3^-] as expected in the defense against respiratory acidosis. In the absence of these responses, the pH falls profoundly, even when the changes in plasma [HCO_3^-] and $PaCO_2$ are

only moderate.

If the respiratory acidosis is less severe than the metabolic acidosis, then the $PaCO_2$ may be normal or even reduced but not to the level appropriate for the respiratory response expected for the metabolic acidosis. If the respiratory acidosis predominates over the metabolic acidosis, then plasma [HCO_3^-] is normal or even increased but not to the level expected for the degree of CO_2 retention, thus indicating a mixed disturbance. Neither change needs to be major in order to cause a significant acidemia. As respiratory treatment is instituted, $NaHCO_3$ may be administered intravenously to treat the metabolic component of the acidosis while the specific etiology and treatment are sought (20).

Respiratory Alkalosis and Metabolic Acidosis

The combination of respiratory alkalosis and metabolic acidosis is seen often in patients with hepatic failure. Such patients may have respiratory alkalosis due to hyperventilation and metabolic acidosis due to renal failure, renal tubular acidosis, liver failure with lactic acidosis, or any combination. Patients with chronic renal failure and metabolic acidosis are susceptible to bacteremia, which may cause increased ventilation and respiratory alkalosis. Salicylate intoxication may cause mixed metabolic acidosis and respiratory alkalosis (74). This combination is a mixed disorder with excessive compensation (Table 4-4). The respiratory alkalosis lowers the $PaCO_2$ beyond the appropriate range of the respiratory response for metabolic acidosis. The plasma [HCO_3^-] also falls below the level expected in simple respiratory alkalosis. In a sense, the compensation for either disorder alone is enhanced; thus, the pH may be normal or close to normal, with a low $PaCO_2$ and a low plasma [HCO_3^-]. The primary therapeutic approach should be directed at treatment of the underlying disorders. The acid–base problem per se usually does not need treatment because the pH is usually closer to normal than it is in either simple disorder alone.

Respiratory Alkalosis and Metabolic Alkalosis

The combination of respiratory and metabolic alkalosis is probably the most common mixed acid–base disorder. This is a mixed disorder with failure of compensation (Table 4-4). It may be seen in patients with

hepatic cirrhosis who hyperventilate, use diuretics, or vomit and in patients with chronic respiratory acidosis and appropriately elevated plasma [HCO_3^-] who are placed on mechanical ventilators and undergo a rapid fall in $PaCO_2$ to hypocapnic levels. Each of the two disorders blocks the appropriate compensatory mechanism of the other; therefore, a marked rise in pH may occur. Depending on the severity of each disorder, the $PaCO_2$ may be normal, reduced, or even increased, whereas the plasma [HCO_3^-] may be normal or elevated. Correction of the metabolic alkalosis by administration of sodium chloride and potassium chloride should be undertaken, and readjustment of the ventilator or treatment of an underlying disorder causing hyperventilation may correct or ameliorate the respiratory disorder.

Metabolic Acidosis and Metabolic Alkalosis

Metabolic acidosis and metabolic alkalosis may coexist in that two separate processes occur sequentially or simultaneously to exert opposing effects on the plasma [HCO_3^-]. A clue to this situation is a deviation in the ratio of change in anion gap to change in bicarbonate. In an anion gap metabolic acidosis, organic acids dissociate into H^+ and the corresponding anion. Therefore, the decrease in serum bicarbonate concentration (due to H^+ buffering) should match the increase in anion gap. A large increase in anion gap associated with a small decrease in bicarbonate suggests a concurrent anion gap metabolic acidosis and metabolic alkalosis. It should be noted that there is some variability in anion gap measurements that precludes a rigid interpretation of this ratio (75). In patients with previous laboratory measurements, interpretation of the "baseline" anion gap can be helpful.

"Triple" Acid–Base Disorders

The occurrence of a primary respiratory disorder in a patient with metabolic acidosis superimposed on metabolic alkalosis results in a "triple" acid–base disorder. That is, three primary processes have acted to alter the acid–base variables. An example is given in Table 4-5. Vomiting raised the plasma [HCO_3^-], which raised the pH, which suppressed ventilation, allowing the $PaCO_2$ to rise a bit. The patient became hypotensive and began to increase lactic acid production and decrease

lactate catabolism, which lowered the [HCO_3^-] from its high level and increased the anion gap well above normal. The hypotension stimulated ventilation beyond that expected for the degree of acidemia, resulting in a further fall in $PaCO_2$, which raised the pH to the alkalemic range. Thus, the high pH and low $PaCO_2$ identify the presence of respiratory alkalosis. The anion gap greater than 27 mEq/L signals the presence of an organic metabolic acidosis. Adding the increase in the anion gap above normal (32 − 9 = 23 mEq/L), which marks the replacement of a HCO_3^- by a metabolic acid anion, to the observed [HCO_3^-] (23 + 14 = 37 mmol/L) indicates the presence of the metabolic alkalosis that raised the [HCO_3^-] in the first place. The serum anion gap is usually the key to the unraveling of triple acid–base disorders. Treatment should be directed at correcting the underlying diseases and replacing volume and electrolyte deficits.

Table 4–5 Example of a Triple Acid–Base Disorder

Clinical Event	Vomiting	→	Hypovolemic Shock	→	Hyperventilation
	↓		↓		↓
Acid–base disorder	Metabolic alkalosis	+	Metabolic acidosis	+	Respiratory alkalosis
pH	7.53		7.35		7.46
$Paco_2$ (mm Hg)	44		30		20
[HCO_3^-] (mmol/L)	36		16		14
Anion gap (mEq/L)	12		30		32

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CHAPTER 5

Disorders of Potassium Metabolism

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Introduction

Potassium plays a key role in maintaining cell function. All cells possess the ubiquitous Na^+/K^+ ATPase exchanger, which pumps Na^+ out of, and K^+ into, the cell. This leads to a K^+ gradient across the cell membrane ($K^+_{in} > K^+_{out}$), which is partially responsible for maintaining the potential difference across the membrane. This potential difference is important to the function of all cells, but is especially important in excitable tissues such as nerve and muscle. For these reasons, the body has developed numerous mechanisms for defense of serum K^+. Total body K^+ is approximately 50 mEq/kg, which in a 70-kg person would be 3,500 mEq. The majority (98%) of this K^+ is within cells with only 2% in the extracellular fluid. The normal concentration of K^+ in the extracellular fluid is 3.5 to 5.3 mEq/L. Large deviations from these values are not compatible with life. Approximately 90% of the daily K^+ intake is excreted in the urine, while 10% is excreted by the gastrointestinal tract.

In 2004, the Food and Nutrition Board of the Institute of Medicine established an adequate intake level for potassium of 4,700 mg/day. Data from NHANES 2007–2008 estimated the mean intake in the United States

to be 2,290 mg/day for women and 3,026 mg/day for men, substantially lower than the recommended value (1). This relative "deficiency" of dietary K^+ is even more noteworthy when one considers that the K^+ intake of prehistoric man was estimated to be 15,000 mg/day, a value that exceeds the NHANES recommendations by a factor greater than 4 (2,3). The mismatch between nutritional requirements encoded into the human genome during evolution and current intake is implicated in various chronic diseases to include hypertension, cardiovascular disease, osteoporosis, and nephrolithiasis. In this regard, dietary supplementation of K^+ has favorable effects in reducing blood pressure, decreasing the risk of stroke, improving bone health, and reducing the risk of nephrolithiasis (4).

The normal kidney can maintain K^+ homeostasis with great facility in the setting of high dietary intake. As an example, serum K^+ levels are maintained in the normal range even when dietary K^+ intake is increased to approximately 15 g daily for 20 days (5). In addition to the well-recognized role of aldosterone in renal K^+ secretion, recent findings have identified the presence of an enteric K^+ sensing mechanism that can initiate the renal secretory process upon K^+ entry into the gastrointestinal tract. Additionally, the distal convoluted tubule (DCT) has been identified as a K^+ sensor capable of initiating K^+ exertion independent of mineralocorticoid activity. Before discussing clinical disorders of K^+ homeostasis, these new insights into renal K^+ handling will be reviewed.

Overview of Renal K^+ Handling

K^+ is freely filtered by the glomerulus and then avidly reabsorbed by the proximal tubule and thick ascending limb such that only a small amount reaches the distal nephron. Reabsorption in the proximal tubule is primarily through the paracellular pathway and is in rough proportion to Na^+ and water. The apical membrane $Na^+/K^+/2Cl^-$ cotransporter mediates transcellular K^+ transport in the thick ascending limb of Henle. In the early DCT K^+ secretion begins and progressively increases in magnitude into the cortical collecting duct. As recently reviewed, the secretory component of K^+ handling is that which varies and is regulated according to physiologic needs (6).

The major K^+-secretory mechanism in the distal nephron is electrogenic secretion through the ROMK (**r**enal **o**uter **m**edullary **K^+**) channel. A second channel (maxi-K^+ or BK channel) also mediates K^+ secretion under conditions of increased flow. In addition to stimulating maxi-K^+ channels, tubular flow also augments electrogenic K^+ secretion by diluting luminal K^+ concentration and stimulating Na^+ reabsorption through the epithelial Na^+ channel (ENaC). In part, this stimulatory effect can be traced to a mechanosensitive property whereby shear stress increases the open probability of the ENaC channel (7).

The biomechanical characteristics of Na^+ and K^+ transport in the distal nephron are ideally suited to buffer any increase in extracellular K^+ concentration following a K^+-rich diet. A protein-enriched meal high in K^+ content typical of early man would lead to an increase in glomerular filtration rate (GFR) and tubular flow (8). Increased flow through the distal nephron increases distal Na^+ delivery and dilutes luminal K^+ concentration both of which augment electrogenic K^+ secretion through the ROMK channel. Along with flow-mediated activation of maxi-K^+ channels, these events enhance renal K^+ secretion thus providing a defense against development of hyperkalemia.

The renal response to a high-K^+, low-Na^+ diet has been studied in experimental rats (9,10). Animals were fed a diet high in K^+ and low in Na^+ for several days and given exogenous deoxycorticosterone to ensure a steady state level of mineralocorticoid activity. Renal K^+ handling was then examined following an acute KCl load. In the initial 2 hours following intraperitoneal injection of KCl, there was a large increase in renal K^+ excretion primarily due to increased secretion into the colleting duct. This increased K^+ secretory capacity is likely due to increased density of both ROMK and maxi-K^+ channels which are both known to increase under conditions of high K^+ intake (11). Over the next 4 hours renal K^+ excretion continued to be high, but the kaliuresis was mostly due to high flow through the collecting duct. The timing of the two phases is important since the effect of high flow to simulate renal K^+ secretion would be maximal only when K^+ channels are maximally expressed.

Increased medullary recycling and accumulation of K^+ in the interstitium of the kidney decreases Na^+ reabsorption both in the thick ascending limb and proximal tubule thus providing a mechanism for high

K^+ intake to increase tubular flow (12,13). Given the high capacity of the proximal tubule and thick ascending limb to reabsorb Na^+, more recent studies have focused on how K^+ intake modulates transport in the low capacity early DCT as a way to adjust tubular flow to K^+ secretory sites. In the setting of a high-K^+, low-Na^+ diet, inhibiting transport in high-capacity upstream segments might lack the precision necessary to ensure delivery of Na^+ appropriate to maximally stimulate K^+ secretion and at the same time not be excessive predisposing to volume depletion.

The Distal Tubule as a K^+ Sensor

The DCT is comprised of a proximal portion (DCT1) where salt transport is driven exclusively by the thiazide-sensitive NaCl cotransporter (NCC) (Fig. 5-1). In the distal part of the DCT (DCT2) electroneutral NaCl transport coexists with electrogenic Na^+ and K^+ transport pathways (14). Aldosterone sensitivity begins in the DCT2 and extends to the collecting duct. Changes in transport in the early DCT control the delivery of NaCl to the downstream connecting tubule and colleting duct where the epithelial sodium channel (ENaC) mediates electrogenic Na^+ reabsorption and where K^+ is secreted. In this regard, cells of the early DCT exert a substantial, albeit indirect, role in K^+ secretion.

The low capacity nature of DCT1 and its location immediately upstream from the aldosterone-sensitive distal nephron (ASDN) makes this segment a more likely site for changes in dietary K^+ intake to modulate Na^+ transport and ensure downstream delivery of Na^+ is precisely the amount needed to ensure maintenance of K^+ homeostasis without causing unwanted effects on volume.

Increased dietary K^+ intake leads to an inhibitory effect on Na^+ transport in the DCT and does so through effects on members of the with no lysine (WNK) family of kinases (15). WNK1 and its shorter spliced variant KS-WNK1 (KS-kidney specific) are highly expressed in the DCT and connecting duct. KS-WNK1 functions as a physiologic antagonist to the actions of long WNK1. Changes in the ratio of KS-WNK1 and long WNK1 in response to dietary K^+ contribute to the physiologic regulation of renal K^+ excretion (16).

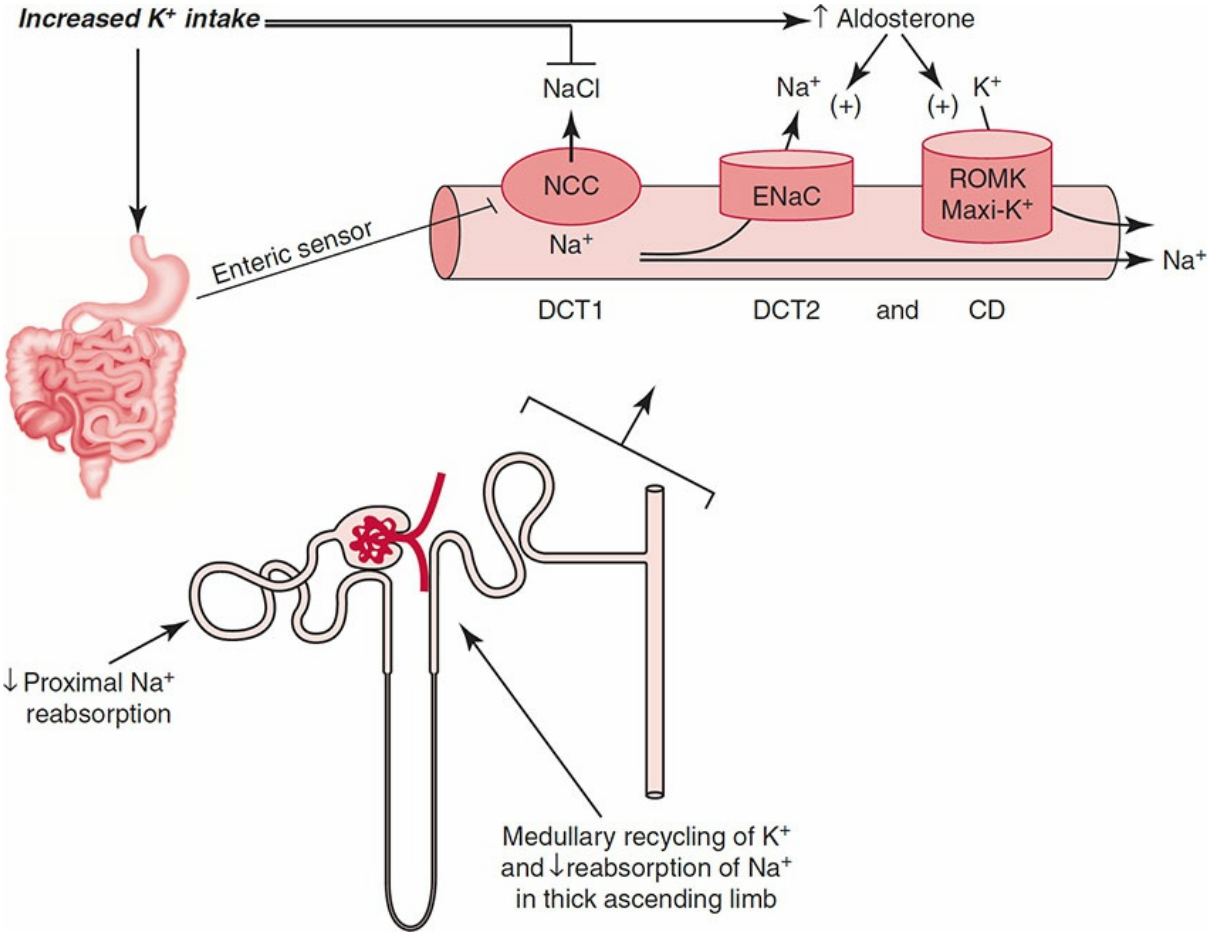

Figure 5–1 Older studies suggest high dietary K^+ intake inhibits Na^+ reabsorption in the proximal nephron and thick ascending limb of Henle causing increased flow and delivery of Na^+ to the aldosterone-sensitive distal nephron resulting in increased K^+ excretion. More recent studies suggest this process is more regionalized to the distal nephron and implicates the distal convoluted tubule (DCT) as a renal K^+ sensor. The proximal portion of the DCT (DCT1) reabsorbs NaCl in an electroneutral fashion via the Na^+/Cl^- cotransporter, NCC. High dietary intake acting through changes in plasma K^+ concentration leads to an inhibitory effect on NCC activity. As a result, Na^+ delivery and flow are increased to the aldosterone-sensitive K^+ secretory segments located in the later portions of the DCT (DCT2) and collecting duct. Increased plasma K^+ stimulates aldosterone release from the adrenal gland which in turn facilitates electrogenic K^+ secretion through ROMK by stimulating. Both increased flow and aldosterone stimulate K^+ secretion through the Maxi-K channel. Increased K^+ secretion may begin upon K^+ entry into the gastrointestinal tract before any change in plasma concentration through an enteric sensing mechanism which leads to an inhibitory effect on NCC activity. DCT, distal convoluted tubule; DCT1, proximal portion of the distal convoluted tubule; NCC, Na^+/Cl^- cotransporter; ENaC, epithelial sodium channel; ROMK, renal outer medullary potassium channel.

Under normal circumstances, long WNK1 prevents the ability of WNK4 (another member of the WNK family) to inhibit activity of the

Na^+/Cl^- cotransporter in the DCT. Thus increased activity of long WNK1 leads to a net increase in NaCl reabsorption. Dietary K^+ loading increases the abundance of KS-WNK1, which blocks the inhibitory effect of long WNK1 on WNK4. The net effect is inhibition of Na^+/Cl^- cotransport in the DCT and increased Na^+ delivery to more distal parts of the tubule. The increase in KS-WNK1 in response to high K^+ intake also antagonizes the effect of long WNK1 to stimulate endocytosis of ROMK. In addition, KS-WNK1 exerts a stimulatory effect on the ENaC. In total, increases in KS-WNK1 in response to dietary K^+ loading facilitates K^+ secretion through the combined effects of increased Na^+ delivery through downregulation of Na^+/Cl^- cotransport in the early DCT increased electrogenic Na^+ reabsorption via the ENaC and greater abundance of ROMK. These effects can occur independent of any change in mineralocorticoid activity suggesting an intrinsic sensing capability of this segment to changes in dietary K^+.

Recent studies suggest extracellular K^+ modulates the WNK axis by altering membrane voltage and changing intracellular chloride concentration (17,18). An increased plasma K^+ concentration, as with increased dietary intake, would depolarize cells in the DCT1 resulting in increased intracellular Cl^- concentration. This increase inhibits activity of WNK4 resulting in decreased activity of NCC. The unique sensitivity of WNK4 to Cl^- is consistent with this model.

High K^+ intake also has a stimulatory effect on release of aldosterone at the level of the adrenal gland. Increased aldosterone complements the effect of KS-WNK1 in the DCT (19,20). Aldosterone upregulates the serum- and glucocorticoid-dependent protein kinase (SGK1) that, in turn, phosphorylates WNK4. This modification prevents WNK4 from inhibiting ROMK and the ENaC (19,21). SGK1 also increases ENaC expression and activity through effects on the ubiquitin-protein ligase Nedd4-2 (22). It should be emphasized that the absence of angiotensin II is a critical factor in the ability of high K^+ intake to bring about the changes necessary to facilitate K^+ secretion without excessive Na^+ reabsorption, a phenomenon referred to as the aldosterone paradox.

K^+-rich foods, such as fruit and vegetables, are also rich in precursors to bicarbonate ions. The alkali present in such a diet directly affects the determinants of K^+ transport in the DCT so as to facilitate the renal excretion of the co-ingested K^+ load (23,24). For example, ENaC

abundance is increased when luminal or basolateral HCO_3^- and pH is elevated. In addition, increased intracellular pH increases activity of ENaC, ROMK, and maxi-K^+ channels. These effects of an alkaline pH provide an additional mechanism to facilitate K^+ excretion following ingestion of such foods.

Enteric Sensing of K^+ Intake

A number of enteric solute sensors capable of responding to dietary Na^+/K^+, and phosphate have been identified which signal the kidney to rapidly alter ion excretion or reabsorption (25,26). In this regard, the ability to sense K^+ within the gastrointestinal tract may have evolved as a way to rapidly initiate the kaliuretic response thereby facilitating maintenance of K^+ homeostasis in the setting of high K^+ intake. For example, the kaliuretic response to a dietary K^+ load is greater when given as a meal compared to an intravenous infusion even in a setting where plasma K^+ concentration is identical. Gastric delivery of K^+ leads to nearly complete dephosphorylation of the Na^+/Cl^- cotransporter in the early DCT causing decreased activity of the transporter thus enhancing delivery of Na^+ to the ASDN (27). The downstream shift in Na^+ reabsorption from the DCT to the ENaC in the ASDN as well as increased maxi-K channel K^+ secretion brought on by increased flow accounts for the increase in renal K^+ excretion. This rapid natriuretic response to increases in dietary K^+ intake is consistent with the blood pressure-lowering effect of K^+-rich diets discussed further below. These data suggest splanchnic sensing of K^+ can initiate the renal excretory response independent of change in plasma K^+ concentration or mineralocorticoid activity.

Hypokalemia

APPROACH TO THE HYPOKALEMIC PATIENT

Hypokalemia is frequently encountered in clinical practice. Transient causes of hypokalemia are due to cell shift while sustained hypokalemia is either due to inadequate intake or excessive K^+ loss. Hypokalemia

resulting from excessive K^+ loss can be due to renal or extrarenal losses. The clinical history, physical examination with particular emphasis on determination of volume status, and determination of the acid–base status will allow the cause of hypokalemia to be readily determined in most cases.

Assessment of renal K^+ excretion allows one to determine whether hypokalemia is due to renal or extrarenal causes. Renal K^+ handling can be assessed with a 24-hour urine collection or a spot urine determining the K^+/creatinine ratio. A 24-hour urinary K^+ of less than 15 mEq or a K^+ (mmol)/creatinine (mmol) ratio less than 1 suggests an extrarenal cause of hypokalemia.

The main limitation to the use of a spot urine K^+ is the influence of renal water handling on urine K^+ concentration. Two patients with similar renal K^+ excretion would have significantly different urine K^+ concentrations depending on whether the urine was concentrated or dilute. The transtubular K^+ gradient (TTKG) has been proposed as a useful tool to assess renal K^+ handling and to overcome this variance.

$$\mathrm{TTKG} = (\mathrm{Urine}_K \times P_{osm})/(P_K \times U_{osm}) \quad (5.1)$$

The K^+ concentration in the final urine will exceed the concentration at the beginning of the collecting duct as a result of water reabsorption along the length of the collecting duct. To account for this effect, the equation divides the urine K^+ concentration by the ratio of the urine osmolality to serum osmolality. In an otherwise normal subject ingesting a typical western diet, the TTKG ranges from 8 to 9 and will increase to >11 with increased K^+ intake. In a chronically hyperkalemic subject, a value <5 would indicate impaired renal K^+ excretion either as a result of aldosterone deficiency or resistance. In patients with hypokalemia due to extrarenal K^+ losses, the TTKG should fall to values <3.

It is worthwhile considering some of the assumptions made in calculating the TTKG. First, the calculation assumes there is no significant solute transport and only water reabsorption as fluid enters the medullary collecting duct. Any Na^+ or urea reabsorption in this segment would tend to lower urine osmolality and cause the TTKG to overestimate the gradient for K^+ secretion in the upstream collecting duct. Second, conditions should be optimal for K^+ secretion at the time the TTKG is measured. In this regard, urine Na^+ should be no less than 25 mEq/L to ensure Na^+ delivery

to the collecting duct is not rate limiting in K^+ secretion. In addition, urine osmolality should be equal to and ideally greater than the plasma. A higher urine osmolality reflects increased vasopressin which is known to exert a stimulatory effect on K^+ secretion in the collecting duct.

While the TTKG is of interest, in most settings, a spot urine K^+ concentration and consideration of the clinical setting will be sufficient to determine the cause of K^+ disturbances. Calculation of the TTKG may prove useful in those patients in which the cause of a dyskalemia continues to remain in doubt (28).

ETIOLOGY OF HYPOKALEMIA

Low K^1 Intake

While the kidney can elaborate urine virtually free of Na^+ in response to dietary Na^+ restriction, the kidney can only reduce urinary K^+ to approximately 15 mEq/day in response to a K^+-free diet. As a result, extreme dietary restriction of K^+ as might occur with a fad diet or eating disorder can conceivably lead to hypokalemia over time. An example of such dietary restriction might occur in patients with anorexia nervosa. Hypokalemia is of particular concern given its association with QT prolongation and ventricular arrhythmias (29). Such changes have been suggested to play a role in the increased risk of sudden death reported in these patients.

More commonly, dietary K^+ restriction simply exacerbates the hypokalemia that is due to other causes. In a recent report, life-threatening hypokalemia developed in a patient with undiagnosed primary hyperaldosteronism after starting a low-carbohydrate, low-calorie diet (30). In addition to less dietary K^+ intake, worsening hypokalemia can also be the result of increased renal K^+ excretion in this setting. Low-carbohydrate diets are accompanied by a period of increased ketogenesis. The excretion of these acids by the kidney in the form of sodium salts results in increased distal delivery of Na^+ in the setting of increased aldosterone. As a result, renal K^+ wasting is exacerbated.

Cellular Redistribution

Since adjustments in renal K^+ excretion can take several hours, changes in

extracellular K^+ concentration are initially buffered by movement of K^+ into or out of the skeletal muscle. The two most important factors that regulate this movement under normal conditions are insulin and catecholamines. For example, following a meal, the postprandial release of insulin not only functions to regulate the serum glucose concentration but also functions to shift dietary K^+ into cells until the kidney excretes the K^+ load reestablishing normal total body K^+ content. During exercise, the release of catecholamines through β_2 stimulation limits the increase in extracellular K^+ concentration that would otherwise occur as a result of the normal K^+ release by the contracting muscle.

Pathologic stimulation of β_2 receptors can result in symptomatic hypokalemia. For example, hypokalemic paralysis is a potential complication of the hyperadrenergic state that oftentimes accompanies alcohol withdrawal syndromes (31). Clenbuterol is a β_2 adrenergic agonist with a rapid onset and long duration of action approved for limited use in veterinary medicine. The drug has been used illicitly as an alternative to anabolic steroids due to its effects to increase muscle mass. Hypokalemia as a result of clenbuterol toxicity has now been reported in users of heroin adulterated with clenbuterol (32).

Intracellular K^+ also serves as a reservoir to limit the fall in extracellular K^+ concentration that occurs under pathologic conditions in which there is loss of K^+ from the body. An example of the efficiency of this buffering effect was previously reported in military recruits undergoing training in the summer (33). The trainees were found to have K^+ losses of >40 mEq/day in sweat alone. At the end of 11 days, subjects exhibited a total body K^+ deficit of approximately 400 mEq and yet the serum K^+ concentration was maintained near normal.

Recent work has been devoted to better define the role of the skeletal muscle in regulating extracellular K^+ concentration (34). In these studies, the movement of K^+ into the skeletal muscle is indirectly determined by the use of a K^+ clamp. With this technique, insulin is administered to rats at a constant rate. K^+ is simultaneously infused at a rate designed to prevent any drop in plasma K^+ concentration. Frequent measurements of plasma K^+ values serve as a guide. The amount of K^+ administered is presumed to be equal to the amount of K^+ entering the intracellular compartment.

This model was used to study the effect of total body K^+ depletion on

insulin-stimulated K^+ uptake. In rats deprived of K^+ for 10 days, the plasma K^+ concentration decreased from 4.2 to 2.9 mmol/L. Insulin-mediated K^+ disappearance declined by more than 90% when compared to control values. This decrease in K^+ uptake was accompanied by a >50% reduction in both the activity and expression of muscle Na^+/K^+ ATPase suggesting that decreased pump activity might account for the decrease in the effect of insulin. When measured after only 2 days of deprivation there was also a significant decline in insulin-mediated K^+ uptake despite the fact that plasma K^+ concentration had only decreased slightly (4.2–3.8 mmol/L). Interestingly, the expression and activity of the Na^+/K^+ ATPase was still normal suggesting the initial resistance to insulin-mediated K^+ uptake is due to a mechanism other than decreased pump activity or expression. This decrease in muscle K^+ uptake under conditions of K^+ depletion may serve to limit an excessive decrease in extracellular K^+ concentration that might otherwise occur under conditions in which insulin is stimulated. These changes would still allow skeletal muscle to buffer any decline in extracellular K^+ concentration by donating some component of its intracellular stores.

Since chronic K^+ depletion decreases skeletal muscle Na^+/K^+ ATPase expression and activity, one would expect that the ability to clear an acute K^+ load under these conditions would be diminished and potentially result in dangerously high levels of plasma K^+. This hypothesis was tested by administering intravenous KCl acutely to rats fed a K^+-free diet for 2 weeks (35,36). In contrast to what was predicted, total K^+ clearance capacity was actually greater in the K^+-depleted animals when compared to K^+-replete controls. The skeletal muscle Na^+/K^+ ATPase pool size was decreased but the decrease was found to be specific to the α-2 isoform which is the major form found in the skeletal muscle. There was no change in the less abundant α-1 isoform. By contrast, cardiac Na^+/K^+ ATPase pool size increased in K^+-deficient animals. Like the skeletal muscle, there was a decrease in the α-2 isoform but a significant increase in the α-1 isoform, which is the dominant form found in the heart. At baseline, the decrease in myocardial K^+ content was considerably less compared to the skeletal muscle. Despite a smaller deficit of K^+, the myocardial uptake of K^+ during the intravenous infusion of K^+ was of the same magnitude as skeletal muscle.

These findings indicate significant differences between skeletal and

cardiac muscle in the response to K^+ depletion. While the skeletal muscle readily relinquishes K^+ to help minimize the drop in plasma K^+ concentration, cardiac tissue K^+ content remains relatively preserved. This difference can be attributed, at least in part, to Na^+/K^+ ATPase isoform differences in the response to K^+ depletion. Cardiac muscle accumulates a considerable amount of K^+ in the setting of an acute load. When expressed on a weight basis, the cardiac capacity for K^+ uptake is comparable to that of the skeletal muscle under conditions of K^+ depletion and may actually exceed the skeletal muscle under control conditions.

Potassium administration in patients with chronic hypokalemia may result in hyperkalemia. This response may be due to the chronic suppression of aldosterone elaboration by hypokalemia. Therefore, in summary, the rate of potassium replacement in the chronic hypokalemic patient should be relatively slow and monitored closely.

Hypokalemic periodic paralysis is a rare disorder that is characterized by muscle weakness or paralysis due to the sudden movement of K^+ into cells. The attacks are precipitated by rest after exercise, stress, high-carbohydrate meals, and events accompanied by increased release of catecholamines or insulin. This disorder may be familial or acquired.

The acquired form of periodic paralysis typically develops in association with thyrotoxicosis (37). Thyrotoxic periodic paralysis is more commonly seen in Asians but has also been reported with higher frequency in Native Americans and Hispanics. While the incidence of thyrotoxicosis is typically more common in women, there is a male to female predominance that ranges from 17:1 to 70:1 in those who developed hypokalemic periodic paralysis. The typical patient is a young adult man age 20 to 40 who presents with weakness most commonly between the hours of 2100 and 0900 during the summer months. The attacks are precipitated by conditions characterized by increased release of catecholamines or insulin such as stress, high-carbohydrate meals, and exercise. With regard to exercise, the timing of attacks is typically in the initial rest period following exertion. Oftentimes the attacks are heralded by muscle cramps and aches and many patients learn to avoid paralytic episodes by exercising the involved muscle groups. Hypophosphatemia and hypomagnesemia are also common during acute attacks and like K^+ is the result of shifts into the intracellular compartment.

Excess thyroid hormone may predispose to paralytic episodes by increasing Na^+/K^+ ATPase activity. The activity of this pump is likely to be increased further by catechols which are typically increased in this

setting. The underlying cause of thyrotoxicosis is most commonly Graves' disease but can also be a solitary thyroid adenoma, a thyroid-stimulating hormone-secreting pituitary adenoma, or abuse of thyroid hormone. Iodine-induced thyrotoxicosis (Jod–Basedow syndrome) and associated hypokalemic periodic paralysis has been reported following the administration of iodine-containing radiocontrast agents, amiodarone, and kelp supplements. "Dream Shape" and "Ever Youth" are two herbal medications used for weight reduction reported to cause iodine-induced thyrotoxicosis without periodic paralysis.

The acute attacks are treated with intravenous KCl and propranolol. It is important to administer KCl in non–dextrose-containing solutions since glucose will stimulate insulin release potentially exacerbating the movement of K^+ into cells. In order to minimize the likelihood of rebound hyperkalemia, K^+ should be given very cautiously and slowly at doses <10 mmol/hour. The goal of potassium administration is not to correct the hypokalemia to the normal range but to increase to a plasma [K^+] of no more than 3.4 mEq/L, and then to discontinue potassium administration in order to avoid severe hyperkalemia, while continuing propranolol as the therapy of choice in this setting. Propranolol (a nonspecific β-adrenergic blocker) blocks the effects of catecholamines and inhibits the peripheral conversion of T_4 to T_3. The definitive treatment is to remove the underlying cause of thyrotoxicosis. Periodic paralysis does not recur once the patient is euthyroid.

The familial form of hypokalemic periodic paralysis is inherited as an autosomal dominant disorder and has similar clinical features as the acquired form. Notable differences include a younger age at presentation (usually <20 years), an equal male-female distribution, and is mostly seen in Caucasians. The familial disorder is most commonly due to mutations in the muscle calcium channel α-1 subunit gene (*CACNA1S*) on chromosome 1q3132. The α-1 subunit of the calcium channel serves as the pore for movement of calcium into the T tubule and also contains the dihydropyridine binding site. Mutations of this subunit reduce the calcium current into the T tubule. The precise mechanism by which impaired function of the calcium channel dihydropyridine receptor causes the influx of K^+ into muscle cells is not entirely clear. A smaller number of cases have been localized to mutations in the skeletal muscle sodium channel *SCN4A* and the R83H mutation in the K^+ channel subunit gene *KCNE3*. Mutations in these genes are not found in patients with thyrotoxic hypokalemic periodic paralysis (38).

The clinical phenotype of patients, together with the pattern of response to therapy, has been shown to differ depending on the location of the mutation. The carbonic anhydrase inhibitor, acetazolamide, is typically an effective therapy in reducing the number of attacks in patients with the familial disorder. The effectiveness of the drug has been attributed to induction of metabolic acidosis, which would in turn limit the intracellular shift of K^+ into cells. However, recent work in an animal model of periodic paralysis suggests the beneficial effects of the drug is actually due to a direct stimulatory effect on Ca^{2+}-activated K^+ channels and not the induction of metabolic acidosis (39). While generally effective, a small number of patients given acetazolamide may demonstrate an exacerbation of symptoms (40). In this regard, the R83H mutation in the K^+ channel subunit gene *KCNE3* reduces single-channel conductance as compared to wild-type channels (41). This decrease in conductance is further reduced under conditions of low pH. It has been suggested that this mutation may explain the tendency for paralytic attacks to occur postexercise since skeletal muscle intracellular pH drops during this period. In addition, this sensitivity to acidosis could provide an explanation for why some patients with this disease worsen after acetazolamide therapy.

Another condition that needs to be considered when evaluating a hypokalemic patient with paralysis is classical distal renal tubular acidosis (dRTA) (42). Muscle paralysis in this disorder can begin insidiously with weakness evolving gradually over a 24- to 48-hour time period to complete flaccid quadriplegia. Attacks of flaccid paralysis in dRTA have been referred to as “RTA crisis” by some authors because this striking clinical manifestation may result in the clinician overlooking the underlying cause. Most of these cases have occurred in patients with dRTA that is idiopathic in origin or as a manifestation of Sjögren’s syndrome in which autoantibody generation prevents the trafficking of the H^+-ATPase to the apical membrane.

Extrarenal K^1 Loss from the Body

Cutaneous loss of K^+ sufficient to cause hypokalemia is uncommon but may occur in the setting of intense exercise in a hot humid environment. Under these conditions, large volumes of sweat can be lost each day. Sweat rates of approximately 2 L/hour were found in a study of football players who were members of a National Collegiate Athletic Association Division II team undergoing preseason training (43). The players practiced

4.5 hours/day and therefore were losing 9 L of sweat per day. This rate of sweat loss was no different whether practices were conducted in half pads or full pads. Body weight only declined between 1 and 2 kg depending on the time of day body weight was measured since the players were allowed free access to water.

A similar sweat volume loss (2.1 L) was found in elite soccer players studied during a single 90-minute preseason training session (44–46). As observed in the football players, body weight declined by a lesser amount (1.2 kg) secondary to fluid intake. Na^+ and K^+ concentration was measured in sweat samples obtained from absorbent sweat pads placed on the chest, forearm, back, and thigh in the soccer players. Sweat Na^+ concentration was 30 mmol/L and total sweat Na^+ loss was 67 mmol. The respective values for K^+ were 3.58 mmol/L and 8 mmol. There are two points worth emphasizing in these studies. First, sweat losses can be substantial in well-trained athletes during exercise. Despite the free availability of water, these athletes remain mildly dehydrated in the immediate postexercise period. Secondly, while the K^+ concentration is low in sweat, significant total body K^+ loss can develop when sweat volume is high.

There is an impression by many that cramps that develop in athletes are related to loss of K^+ in sweat. To address this issue sweat Na^+ and K^+ losses were measured in Division I collegiate football players undergoing twice-daily practice sessions (47). Values obtained were compared between players with a history of heat cramps (C) and teammates with no history of cramping (NC). In a 2.5-hour practice session, sweat loss was similar between the two groups (4.0 l [C] vs. 3.5 l [NC]). Sweat K^+ was also similar in the two groups but sweat Na^+ was two times higher in players with a history of heat cramps (54 vs. 25 mmol/L). These data suggest it is large acute Na^+ and fluid loss rather than K^+ loss that is associated with a tendency to develop cramps during exercise.

Gastrointestinal loss is a common cause of hypokalemia and is generally due to diarrhea. Secretory diarrhea is generally believed to be caused by one of two processes which can either occur alone or both together (48,49). First, there can be inhibition of active intestinal NaCl and $NaHCO_3$ reabsorption and second, there can be stimulation of active chloride secretion which is then followed by passive secretion of an equal amount of Na^+ so as to maintain electrochemical balance. In both of these instances, the stool electrolyte content is similar to plasma with high concentration of NaCl and much lower K^+ concentration. The sodium salts

in stool cause an isotonic increase in stool water output such that the fecal content of sodium salts roughly parallels the volume of diarrhea. Despite the low K^+ concentration in fecal fluid, significant total body K^+ losses can occur in the setting of large stool volumes.

Severe K^+ depletion due to secretory diarrhea has been the subject of recent case reports. One patient with neurofibromatosis type 1 presented with the syndrome of watery diarrhea, hypokalemia, and achlohydria due to oversecretion of vasoactive intestinal polypeptide (VIP) (50). A neuroendocrine tumor composed of pheochromocytoma and ganglioneuroma was responsible for the VIP production. A second patient presented with hypokalemia complicated by rhabdomyolysis due to oversecretion of pancreatic polypeptide (PP) from a pancreatic islet tumor (51). PP normally exerts an inhibitory effect on gastric emptying and upper gastrointestinal motility but has been reported to cause a watery diarrhea syndrome similar to that as a VIPoma. In this patient severe hypokalemia developed with minimal gastrointestinal complaints. The authors speculated that the inhibitory effect of PP on gut motility may have attenuated the development of watery diarrhea. Hypokalemia and volume depletion can also occur in association with villous adenomas, a symptom complex referred to as the McKittrick–Wheelock syndrome (52).

Abnormalities in K^+ transport have not previously been known to be the primary cause of secretory diarrhea. The first such case has recently been described in an elderly woman with colonic pseudo-obstruction (Ogilvie's syndrome) (53). One week after undergoing surgical treatment of a hip fracture, a 78-year-old woman developed diarrhea, hypokalemia, and a markedly dilated colon. The workup excluded identifiable causes of intestinal obstruction and diarrhea. Fecal fluid was collected on multiple occasions and electrolyte content was measured. In contrast to the high Na^+ concentration (101–137 mEq/L) and low K^+ concentration (16–51 mEq/L) typically found in various causes of secretory diarrhea, fecal electrolyte concentration in this patient was reversed. Fecal K^+ concentration ranged from 130 to 170 mEq/L while values for Na^+ concentration varied between 4 and 15 mEq/L. In addition, increased stool weight was accompanied by a proportionate increase in fecal K^+ output while stool Na^+ changed very little. Measurement of the rectosigmoid potential difference in this patient was −13 mV (lumen negative). When the data were interpreted in the context of the Nernst equation, the authors concluded there was evidence of active K^+ secretion along with active Na^+ reabsorption across the colonic mucosa. The patient's pseudo-obstruction

spontaneously resolved over a 14-week period.

As in the kidney, K^+ is actively absorbed and secreted in the colon whereas in the small intestine K^+ movement is strictly diffusional. Colonic K^+ absorption occurs via the colonic isoform of H^+/K^+ ATPase, located on the luminal membrane, while K^+ secretion occurs via a luminal channel (54,55). Dietary K^+ restriction increases colonic K^+ absorption while secretion predominates with high dietary K^+ intake. In patients with end-stage renal disease, increased secretion of K^+ by the colon is an important adaptation to K^+ homeostasis. Recent studies indicate colonic K^+ secretion is mediated by the BK channel (56). This channel is a high conductance channel that is activated by calcium and is located exclusively in the colonic crypts and not on surface cells. Increased expression of this channel may mediate the enhanced colonic K^+ secretion in patients with end-stage renal disease.

Bowel cleansing solutions can occasionally be associated with perturbations in serum electrolyte levels to include hypokalemia, hyperphosphatemia, hypocalcemia, and hypernatremia. Risk factors for these disturbances include advanced age, the presence of bowel obstruction, poor gut motility, and unrecognized renal disease. Use of oral sodium phosphate for bowel preparation has recently been linked to the development of acute renal failure characterized histologically by widespread deposition of calcium phosphate crystals (57). A recent study randomly allocated 100 consecutive patients who were to undergo colonoscopy to either receive sodium phosphate or a glycol-electrolyte solution (Golytely, Braintree Laboratories) for bowel cleansing (58). Eleven patients received sodium phosphate despite the presence of factors that would have identified them as being at risk for complications. In six of these high-risk patients, the serum phosphate doubled and hypokalemia developed in four. In subjects without risk factors, mild hyperphosphatemia developed in 39% and hypocalcemia developed in 5%. In the glycol-electrolyte solution-treated patients, only mild and clinically insignificant changes in serum electrolyte values occurred. The glycol-electrolyte solution is a nonabsorbable and osmotically balanced solution and has virtually no net effect on electrolyte absorption or secretion.

Severe hypokalemia can result from K^+ binding in the gastrointestinal tract. A serum K^+ concentration of 0.9 mmol/L was found in a 3-year-old girl following several days of oral and rectal administration of bentonite given as a home remedy for the treatment of constipation (59). Bentonite, also called montmorillonite or fuller’s earth, is a type of clay primarily

composed of hydrated aluminum silicate. Clay eating (geophagia) can be a manifestation of pica and has been reported to cause hypokalemic paralysis during pregnancy and in the postpartum period (60,61).

Renal Potassium Wasting

The elaboration of aldosterone and distal delivery of Na^+ and water are two important factors in the renal excretion of K^+. Although increased distal delivery of Na^+ and water and increased aldosterone activity can each stimulate renal K^+ secretion, under normal physiologic conditions, these two determinants are inversely related. It is only under pathophysiologic conditions that distal Na^+ delivery and aldosterone become coupled. In this setting, renal K^+ wasting will occur (Fig. 5-2). When treating patients who are hypokalemic as a result of renal K^+ wasting, it must be determined whether there is a primary increase in mineralocorticoid activity or a primary increase in distal Na^+ delivery (62).

PRIMARY INCREASE IN MINERALOCORTICOID ACTIVITY

Increases in mineralocorticoid activity can be due to primary increases in renin secretion, primary increases in aldosterone secretion, or increases in a non-aldosterone mineralocortiocid or increased mineralocorticoid-like effect. In all of these conditions, extracellular fluid volume is expanded and hypertension is typically present. The differential diagnosis for the patient with hypertension, hypokalemia, and metabolic alkalosis rests on the renin and aldosterone levels (Fig. 5-3).

Increased Renin, Increased Aldosterone

Renin-secreting tumors are most commonly hemangiopericytomas. These tumors are highly vascular and arise from the pericapillary pericytes of Zimmerman and postcapillary venules. These tumors have also been associated with the development of oncogenic osteomalacia due to overproduction of fibroblast growth factor 23 and the Kasabach–Merritt syndrome. This syndrome describes the presence of a consumptive coagulopathy in which thrombocytopenia is a major feature. Renal artery stenosis can be associated with activation of the renin–angiotensin–aldosterone cascade as a result of renal ischemia. Hypokalemia is seen in

approximately 15% of cases.

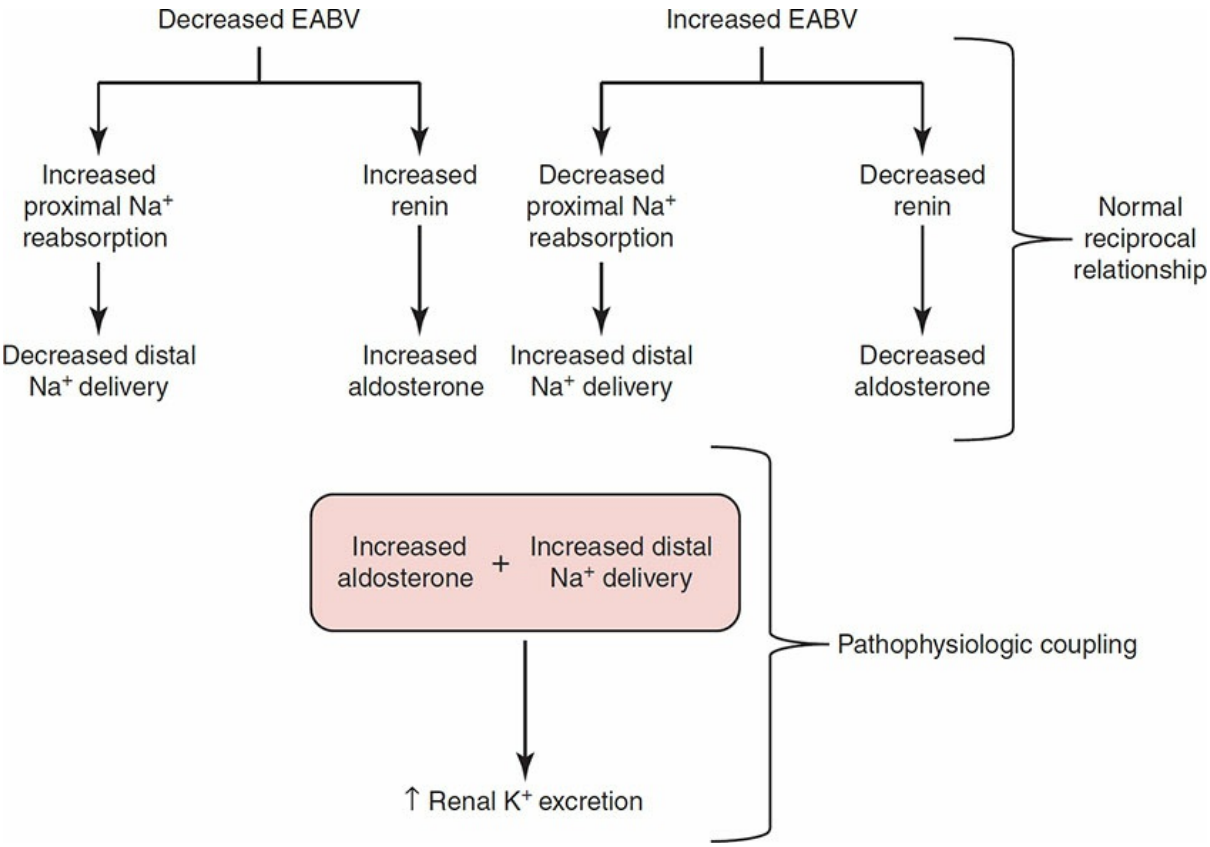

Figure 5–2 Under normal circumstances delivery of Na^+ to the distal nephron is inversely associated with serum aldosterone levels. For this reason, renal K^+ excretion is kept independent of changes in extracellular fluid volume. Hypokalemia due to renal K^+ wasting can be explained by pathophysiologic changes that lead to coupling of increased distal Na^+ delivery and aldosterone or aldosterone-like effects.

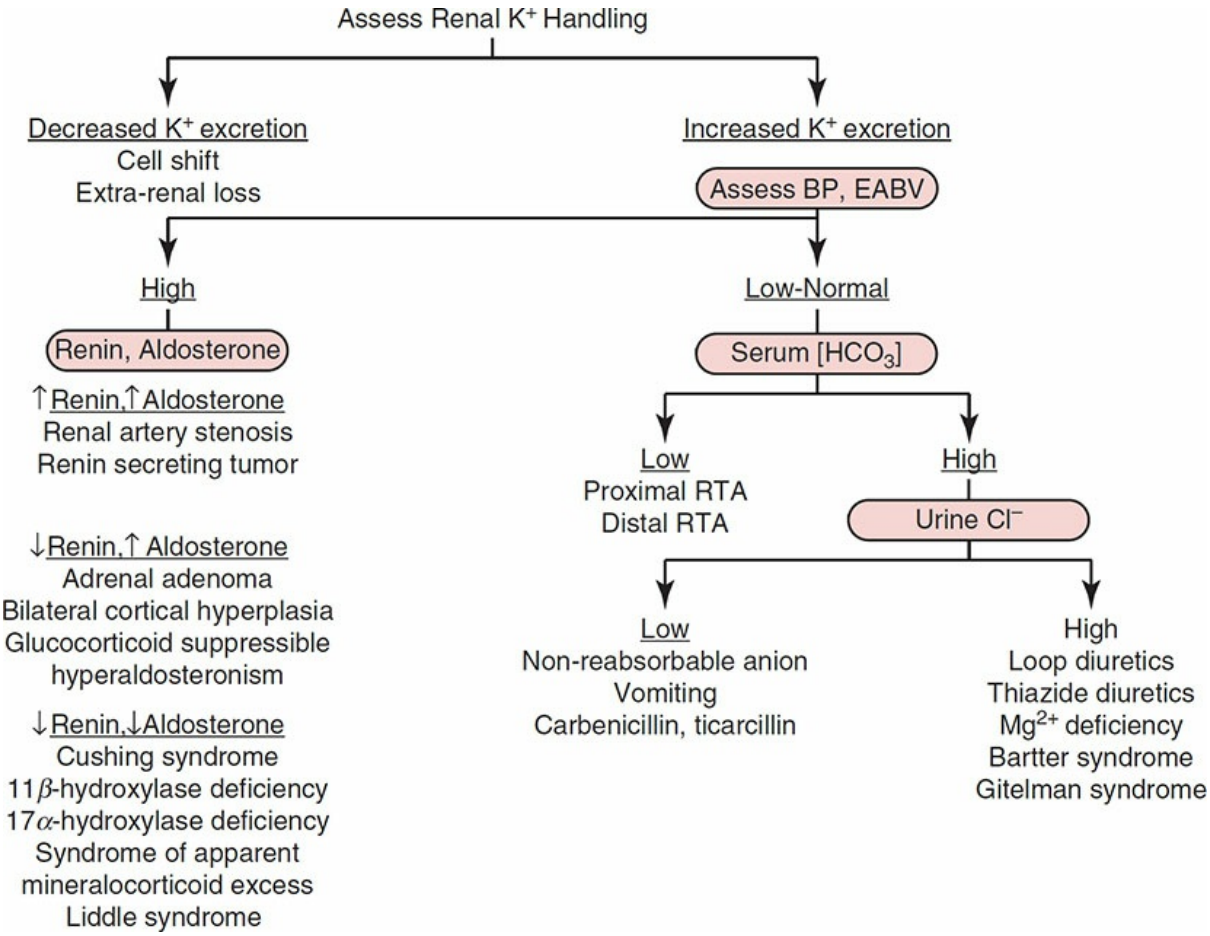

Figure 5–3 Diagnostic approach to the patient with hypokalemia.

Suppressed Renin, Increased Aldosterone

The most common disease in this category is primary aldosteronism due either to an adrenal adenoma or bilateral adrenal hyperplasia. The most common screening test used to detect this entity is measurement of the ratio of plasma aldosterone concentration to plasma renin activity. The mean value of this ratio in normals or patients with essential hypertension is 4 to 10. Patients with primary hyperaldosteronism have values of 30 to 50 (63). If this test is positive, then confirmatory testing is indicated. Intravenous saline loading, oral sodium loading, or the fludrocortisone suppression test can be used for this purpose (64).

Glucocorticoid-remediable aldosteronism (GRA) is the most common monogenic cause of human hypertension. The disease is inherited in an autosomal dominant fashion and is characterized by adrenocorticotropin (ACTH)-dependent aldosterone secretion. GRA results from the unequal crossover of two genes: the *CYP11B1* gene that encodes the enzyme 11β-hydroxylase and the *CYP11B2* gene that encodes the enzyme aldosterone

synthase (18-hydroxylase). The product of this event creates a chimeric gene in which the ACTH-responsive promoter is fused to the aldosterone synthase coding sequence. As a result, aldosterone synthase is ectopically expressed in the cortisol-producing zone of the adrenal cortex (zona fasciculata) and is under the control of ACTH. Suppression of aldosterone with exogenous administration of dexamethasone is a useful diagnostic and therapeutic strategy. Measurement of urinary cortisol metabolites can also be useful as a diagnostic tool (65). Increased urinary excretion of 18-hydroxycortisol is typical of this disease.

For unclear reasons, random K^+ levels are frequently normal in patients with GRA. One possibility may be that the stimulatory effect on aldosterone release is only intermittent since ACTH is secreted centrally in a diurnal fashion with peaks in the early morning and evening. GRA patients have been noted to develop hypokalemia frequently when treated with thiazide or loop diuretics.

Suppressed Renin and Aldosterone

Cushing's disease or syndrome is the most common disease within this category. The clinical manifestations of Cushing's disease result from chronic exposure to excess glucocorticoids. Such manifestations include moon facies, abdominal obesity, buffalo hump, and striae. Fluid and electrolyte manifestations include hypertension and hypokalemic alkalosis. High concentrations of cortisol overwhelm the ability of the kidneys to convert cortisol to cortisone and result in activation of the mineralocorticoid receptor.

There are monogenic forms of hypertension characterized by suppressed circulating levels of renin and aldosterone (66). 11β-Hydroxylase deficiency is an autosomal recessive disorder that prevents the production of cortisol. The lack of feedback control results in high ACTH levels which in turn drives the synthesis of 11-deoxycortisol and 11-deoxycorticosterone. These compounds have mineralocortiocid-like effects and cause Na^+ retention and renal K^+ wasting. Precursors of cortisol are also driven to increased production of androgens so that such patients have clinical features of virilization.

17α-Hydroxylase deficiency is a rare disorder that also interferes in the synthesis of cortisol. As with 11β-hydroxylase deficiency, hypertension and hypokalemia develop due to the accumulation and mineralocorticoid-like effects of 11-deoxycortisol and 11-deoxycorticosterone. This disorder is also accompanied by a reduction in androgen production and estrogen

deficiency.

The reabsorption of Na^+ across the apical membrane in the collecting duct occurs through an amiloride-sensitive Na^+ channel, ENaC, that is formed by the assembly of three subunits: *α*, *β*, and *γ*. Liddle's syndrome is an inherited form of hypertension and hypokalemic metabolic alkalosis caused by mutations in this channel. These mutations either delete or alter residues in the C-terminal PY motif of the *β* and *γ* subunits. The PY motif of *α*, *β*, and *γ* subunits is the binding site for the WW domain of a cytoplasmic protein called Nedd4-2. The binding of Nedd4-2 to the PY motif on each of the three subunits leads to ubiquitination of the epithelial sodium channel tagging it for eventual endocytosis and degradation. Interference in PY motif binding leads to an inability to retrieve the channel from the membrane and results in increased channel density, which gives rise to the clinical characteristics suggesting constitutive activation of the epithelial sodium channel (22,67,68).

In addition to increased surface density there is evidence that Liddle's mutations may also enhance Na^+ transport by increasing the open-state probability of ENaC (69). Under normal conditions ENaC exists at the cell surface in two distinct pools. In one pool, the extracellular domain of the subunits is proteolytically cleaved resulting in channels with a high open probability. A second pool is uncleaved and has a low open-channel probability and/or conductance. Nedd4-2 has the effect of reducing the fraction of cleaved (active) channels at the cell surface. By contrast, Liddle's syndrome mutations have the opposite effect. Thus, the increase in Na^+ current in Liddle's syndrome is the result of a generalized increase in ENaC expression along with a greater fraction of cleaved channels in the open state.

ENaC activity is also regulated by a negative feedback mechanism sensitive to intracellular Na^+. As intracellular Na^+ rises, there is an inhibitory effect on further Na^+ entry via ENaC. Under normal conditions this inhibitory effect is mediated by a reduction in channel density as well as a decrease in open-channel probability. This effect has now been studied in the ENaC harboring a *β*-subunit mutation associated with Liddle's syndrome (70). As compared to wild-type channels, Liddle's mutants are less sensitive to the inhibitory effect of intracellular Na^+. In addition, the reduction in Na^+ entry that does occur is solely mediated by a decrease in open-channel probability with no change in channel density.

The syndrome of apparent mineralocorticoid excess is a rare recessive disorder characterized by hypertension, hypokalemia, metabolic alkalosis,

and suppressed circulating aldosterone levels. This disease is due to decreased activity of the 11β-hydroxysteroid dehydrogenase enzyme type II. This enzyme is normally found in aldosterone-responsive cells and functions to protect these cells from inappropriate activation of the mineralocorticoid receptor by circulating cortisol.

The mineralocorticoid receptor is capable of binding cortisol and aldosterone with equal affinity. Cortisol circulates in the blood at a concentration that is greater than 1,000-fold higher than aldosterone. Despite these higher concentrations, cortisol is prevented from binding to the mineralocorticoid receptor because it is rapidly converted to cortisone by 11β-hydroxysteroid dehydrogenase type 2. The enzyme allows aldosterone, rather than cortisol, to gain access to the receptor despite the fact that circulating levels of aldosterone are much lower. A decrease in the activity of this enzyme removes the selectivity for aldosterone and allows cortisol to persistently activate the mineralocorticoid receptor thus accounting for the development of clinical manifestations. Patients with this disorder can be effectively treated with either spironolactone or a sodium channel blocker such as amiloride or triamterene.

Decreased activity of 11β-hydroxysteroid dehydrogenase type 2 can occur as an acquired disorder due to the chronic ingestion of licorice. The active component in licorice is glycyrrhetinic acid that has an inhibitory effect on the enzyme. Licorice is used as a flavoring agent in a variety of products such as chewing tobacco. Other agents containing glycyrrhetinic acid reported to cause this syndrome include herbal medications used to treat allergic rhinitis and constipation and the flavoring product “Asam Boi” widely consumed by the Malaysian and Singapore populations (71). Flavonoids found in grapefruit juice have also been shown to have an inhibitory effect on the enzyme (72).

Acquired inhibition of 11β-hydroxysteroid dehydrogenase type 2 may be of importance in the salt retention that occurs in some patients with cirrhosis. Aldosterone is generally believed to play a major role in the renal salt retention observed in cirrhotic patients. However, there are many examples of patients who present with ascites and total body sodium overload who have either normal or suppressed aldosterone levels. Bile acids which can accumulate in the setting of chronic liver disease have been shown to inhibit the activity of 11β-hydroxysteroid dehydrogenase type 2 (73). Such an effect would allow cortisol-mediated stimulation of the mineralocorticoid receptor and potentially explain aldosterone-independent salt retention in the distal nephron (74).

An autosomal dominant form of hypertension that results from an

activating mutation (S810L) in the mineralocorticoid receptor has been described in a single kindred. The development of hypertension is associated with hypokalemia and suppressed serum aldosterone levels. Hypertension typically develops before the age of 20 in affected family members. In addition, there is a marked worsening of hypertension that occurs during pregnancy.

Steroids with a 21-hydroxyl group such as aldosterone and cortisol are capable of activating both the wild type and mutant receptor. The crystal structure of the wild type and mutant receptors has recently been identified (75). Under normal conditions steroids that lack the 21-hydroxyl group but containing a 17-keto group are mineralocorticoid receptor antagonists because they bind but do not activate the normal receptor. By contrast, the mutated receptor is activated by these steroids. Progesterone (a 17α hydroxyl steroid) lacks the 21-hydroxyl group and is capable of activating the mutated receptor thus explaining the worsening of hypertension that occurs during pregnancy when progesterone levels are increased. Spironolactone (a synthetic steroid with a 17γ lactone) also activates the mutated receptor and therefore should be avoided in this condition. Cortisone is also capable of activating the mutated receptor and has been implicated in the development of hypertension in young men and nonpregnant women harboring the S810L mutation (76). The sodium channel blockers amiloride or triamterene are treatment options.

Primary Increase in Distal Na^+ Delivery

Conditions that give rise to primary increases in distal Na^+ delivery are characterized by normal or low extracellular fluid volume. Blood pressure is typically normal. Increases in distal Na^+ delivery are most frequently due to diuretics which act proximal to the cortical collecting duct. Increased delivery can also be the result of nonreabsorbed anions such as bicarbonate as with active vomiting or a type II proximal renal tubular acidosis. Ketoanions and the Na^+ salts of penicillins are other examples. The inability to reabsorb these anions in the proximal tubule results in increased delivery of Na^+ to the distal nephron. Since these anions also escape reabsorption in the distal nephron, a more lumen-negative voltage develops and the driving force for K^+ excretion into the tubular fluid is enhanced.

Disorders of hypokalemia due to primary increases in distal Na^+

delivery can best be categorized as to the presence of metabolic acidosis or metabolic alkalosis (Fig. 5-3). Falling into the category of metabolic acidosis are disorders that cause renal tubular acidosis. In proximal renal tubular acidosis the threshold for bicarbonate reabsorption is reduced resulting in a self-limited bicarbonaturia. The loss of $NaHCO_3$ in the urine leads to volume depletion, which in turn activates the renin–angiotensin–aldosterone system (RAAS). The coupling of increased aldosterone levels to increased distal Na^+ delivery results in renal K^+ wasting. In the steady state when virtually all the filtered HCO_3 is reabsorbed in the proximal and distal nephron, renal K^+ wasting is minimal and the degree of hypokalemia tends to be mild. By contrast, treatment of metabolic acidosis with bicarbonate improves the acidosis but worsens the degree of hypokalemia.

The development of hypokalemia in dRTA can be due to several mechanisms. First, systemic acidosis in and of itself can lead to renal K^+ wasting (23). Metabolic acidosis is associated with decreased net proximal Na^+ reabsorption. The subsequent increase in distal delivery leads to volume contraction and activation of the RAAS. These changes lead to increased renal K^+ excretion. Second, dRTA due to a defect in the H^+/K^+ ATPase will increase renal K^+ excretion by directly impairing K^+ reabsorption in the distal nephron. Third, K^+ wasting can be the result of leakage into the tubular lumen as a result of an ionophoric effect as seen in the gradient type of dRTA due to administration of amphotericin B.

Hypokalemia can be severe and potentially life threatening in dRTA. Recent reports have described profound hypokalemia complicated by paralysis in patients with Sjögren's syndrome (77). While this disorder typically occurs in middle-aged women, it can also be seen in elderly men. A clue to the diagnosis of dRTA can be the discovery of nephrocalcinosis visualized on a normal lung radiograph (78).

Toluene inhalation can give rise to severe and symptomatic hypokalemia in association with a hyperchloremic normal gap metabolic acidosis. These electrolyte derangements are largely due to production of hippuric acid (a metabolite of toluene) and the renal excretion of its sodium salt. Chronic exposure may lead to a persistent dRTA due to direct tubular toxicity.

Falling in the category of hypokalemia and metabolic alkalosis is use of loop diuretics and Bartter syndrome. Bartter syndrome is a hereditary disorder characterized by renal salt wasting and hypokalemic metabolic alkalosis resembling the features of chronic loop diuretic therapy. Hypokalemia can be severe and result in complications such as

rhabdomyolysis and periodic paralysis (79,80). This disease results from gene defects that lead to decreased NaCl reabsorption in the thick ascending limb of Henle.

The development of hypokalemia in type II Bartter syndrome illustrates the importance of maxi-K^+ channels in renal K^+ excretion (81). These patients have a loss-of-function mutation in ROMK and present with clinical features of the disease in the perinatal period. ROMK provides the pathway for recycling of K^+ across the apical membrane in the thick ascending limb of Henle. This recycling generates a lumen-positive potential, which drives the paracellular reabsorption of Ca^{2+} and Mg^{2+} and provides luminal K^+ to the $Na^+/K^+/2Cl^-$ cotransporter. Mutations in ROMK decrease NaCl and fluid reabsorption in the thick limb mimicking a loop diuretic effect causing volume depletion. Despite the increase in distal Na^+ delivery, one would not predict the development of renal K^+ wasting since ROMK is also the major K^+-secretory pathway for regulated K^+ excretion in the collecting duct. In fact, in the perinatal period, infants with this form of Bartter syndrome often exhibit a transient hyperkalemia consistent with loss of function of ROMK in the collecting duct. However, over time, these patients develop hypokalemia as a result of increased flow-mediated K^+ secretion via maxi-K^+ channels. Studies in a ROMK-deficient mouse model of type II Bartter syndrome are consistent with this mechanism (82). The transient hyperkalemia observed in the prenatal period is likely related to the fact that ROMK channels are functionally expressed sooner than maxi-K^+ channels.

Gitelman syndrome is an inherited disorder with clinical manifestations that mimic the chronic use of a thiazide diuretic, including hypokalemia, hypomagnesemia, and hypocalciuria. This disease is due to an inactivating mutation in the gene (*SLC12A3*) for the thiazide-sensitive apical NaCl cotransporter (NCC) in the DCT. Immunohistochemistry performed on renal biopsy material taken from two adults with Gitelman syndrome were devoid of intact NCC immunostaining (83). Although the disease may be relatively benign with either no or minimal symptoms, many patients have difficult hypokalemia and represent a therapeutic challenge. One explanation for difficulty with management with K^+ supplementation, amiloride, and spironolactone is that patients with Gitelman syndrome often manifest salt craving. High dietary salt and fluid intake increase distal Na^+ delivery and drive K^+ secretion by the unaffected cortical collecting tubule (CCT) principal cell. Paralysis and prolongation of the QT interval with malignant arrhythmias attributed to

hypokalemia and hypomagnesemia have been described in patients with the features of Gitelman syndrome (84,85).

Complications and Treatment of Hypokalemia

Hypokalemia can cause a variety of clinical manifestations due to alterations in the excitability of neuromuscular tissues. Decrease in extracellular K^+ concentration leads to hyperpolarization of the cell membrane, causing the cell to become less sensitive to exciting stimuli. Clinically, this effect accounts for the association of hypokalemia and muscle weakness. Occasionally, muscle weakness can be sufficiently severe to cause paralysis as occurs in patients with hypokalemic dRTA secondary to Sjögren's syndrome (86).

Under normal circumstances, exercise is associated with movement of intracellular K^+ into the interstitial space in skeletal muscle. The increase in interstitial K^+ can be as high as 10 to 12 mmol with extreme exertion. This accumulation of K^+ has been implicated as a factor limiting the excitability and contractile force of muscle accounting for the development of fatigue (87,88). In addition, increases in interstitial K^+ are thought to be an important factor in eliciting rapid vasodilation, allowing for blood flow to increase in the exercising muscle (89). Hypokalemia is a cause of rhabdomyolysis. Although the mechanism is likely to be multifactorial, total body K^+ depletion may blunt the accumulation of K^+ into the interstitial space, thereby limiting blood flow to the skeletal muscle and resulting in muscle breakdown.

Hypokalemic nephropathy or "kaliopenic nephropathy" is a chronic tubulointerstitial disease characterized by polyuria, proteinuria, development of renal cysts, and loss of renal function. Histologically, there is evidence of tubular atrophy, interstitial infiltration of macrophages, and interstitial fibrosis. Mediators of renal injury in this setting include local ischemia, complement activation due to increased ammoniagenesis, and local effects of angiotensin II and endothelin. Studies in Sprague–Dawley rats fed a low K^+ diet implicate impaired angiogenesis as an additional mechanism of renal injury in this disorder (90).

Hypokalemia may play a role in the association between new onset diabetes and use of thiazide diuretics. In a recent review of more than 50 trials in which thiazides were compared with other drugs or placebo, a significant inverse relationship was found between the decrease in K^+ and

the increase in glucose level (91). For every 1 mEq/L decrease in K^+, there was an approximate 10 mg/dL increase in glucose. Further strengthening the argument that hypokalemia plays an important role in the genesis of glucose intolerance is the observation that prevention of hypokalemia with K^+ supplements prevents the development of thiazide-induced glucose intolerance (92). In addition, changes in glucose levels can be normalized following K^+ repletion in patients who are hypokalemic.

The mechanism of thiazide-induced hyperglycemia is thought to be the result of decreased insulin released from the pancreatic beta cell. ATP-sensitive K^+ channels couple beta-cell metabolism to electrical activity, thereby playing an essential role in the control of insulin secretion (93). The involvement of K^+ in this process at least raises the possibility that K^+ depletion might alter beta cell insulin release. Impaired insulin release that is reversible with drug discontinuation or K^+ supplements is in contrast to the persistent insulin resistance typical of patients with type II diabetes. This difference in the mechanism of glucose intolerance may help explain the lack of convincing evidence that thiazide-induced diabetes mellitus increases the incidence of morbid or fatal cardiovascular events (94).

In the hypokalemic patient, K^+ can be given orally or intravenously as the KCl salt. Because $KHCO_3$ and potassium citrate are converted to bicarbonate, increase the bicarbonate concentration in plasma, and enhance HCO_3^- excretion, these products may increase K^+ excretion and are therefore not the replacement of choice except for patients with concomitant metabolic acidosis. Oral administration of KCl is safer and more effective. KCl can be given in doses of 100 to 150 mEq/day. Liquid KCl is bitter tasting, and the tablet can be irritating to the gastric mucosa. The microencapsulated or wax-matrix forms of KCl are better tolerated.

Intravenous administration of K^+ may be necessary if the patient cannot take oral medications, or if the K^+ deficit is large and is resulting in cardiac arrhythmias, respiratory paralysis, or rhabdomyolysis. Intravenous KCl should be given at a maximum rate of 20 mEq/hour and maximum concentration of 40 mEq/L. Higher concentrations will result in phlebitis. Replacement of KCl in dextrose-containing solutions can result in further lowering of the serum K^+ secondary to insulin release. Thus, saline solutions are preferred.

On rare occasions, higher concentrations of K^+ may have to be given. In a patient with a serum K^+ of 2.6 mmol/L and an implantable cardiac defibrillator, rapid administration of K^+ successfully led to the termination

of recurrent unstable ventricular tachycardia (95). This patient was given a rapid bolus of 20 mEq KCl solution through a central access, followed by an additional 80 mEq orally and intravenously during the next 2 h. A 12-year-old boy with a K^+ of 1.2 mEq/L due to gastrointestinal losses was given 140 mEq of KCl as a bolus after developing pulseless ventricular tachycardia (96). The bolus administration leads to resolution of the arrhythmia. Aggressive K^+ administration requires frequent measurement of serum K^+ and continuous electrocardiographic monitoring to prevent iatrogenic hyperkalemia. In a retrospective survey of 140 hospitalized patients with hypokalemia, 16% of patients developed therapy-induced hyperkalemia. Compared with patients who simply corrected to normal, the amount of K^+ given was greater for patients with iatrogenic hyperkalemia (97). Therefore, careful administration and frequent monitoring of plasma [K^+] is required.

Hyperkalemia

PSEUDOHYPERKALEMIA

Pseudohyperkalemia is an in vitro phenomenon due to the mechanical release of K^+ from cells during the phlebotomy procedure or specimen processing. This diagnosis is made when the serum K^+ concentration exceeds the plasma K^+ concentration by >0.5 mmol/L. Common causes of pseudohyperkalemia include fist clenching during the phlebotomy procedure, application of tourniquets, and use of small-bore needles. Pseudohyperkalemia can also occur when specimens are transported via a pneumatic tube transport system (98).

The presence of a high cell or platelet count is another setting where pseudohyperkalemia is frequently observed. Differences in serum and plasma K^+ concentration were recently compared in patients with increased cellular components of blood of various causes (99,100). As compared to normal controls, the delta serum plasma K^+ concentration was significantly increased in patients with erythrocytosis and thrombocytosis but not in patients with white blood cell disorders. The delta was particularly pronounced in subjects with polycythemia vera with both an increase in red blood cell and platelet counts.

Familial pseudohyperkalemia is a symptomless genetic disorder of red blood cell membrane permeability in which measurement of K^+ is normal

at the time of blood collection but is increased when measured after the sample has been allowed to stand at room temperature for several hours. This temperature-dependent leakage of K^+ out of the cell is inherited in an autosomal dominant fashion and has only been described in several kindreds. In one of these kindreds, the disorder maps to the same loci as patients with hereditary stomatocytosis.

EXCESSIVE DIETARY INTAKE

In the presence of normal renal and adrenal function, it is difficult to ingest sufficient K^+ to develop hyperkalemia. Rather, dietary intake as a contributor to hyperkalemia is usually in the setting of impaired kidney function. Dietary sources particularly enriched with K^+ include melons, dried fruits, citrus juice, and salt substitutes. Other hidden sources of K^+ reported to cause life-threatening hyperkalemia include raw coconut juice (K^+ concentration of 44.3 mmol/L) and Noni juice (101,102). While clay ingestion can cause hypokalemia due to binding in the gastrointestinal tract, river bed clay is K^+ enriched (100 mEq K^+ in 100 g clay) and can cause life-threatening hyperkalemia in chronic kidney disease (CKD) patients (103). Ingestion of burnt match heads (cautopyreiophagia) can also be a hidden source of K^+ (104). This activity was found to add an additional 80 mmol of K^+ to one dialysis patient's daily intake and produced a plasma K^+ concentration of 8 mmol/L.

K^+ supplements need to be used with caution even in patients with renal K^+ wasting disorders since they may develop loss of kidney function due to unrelated reasons. This scenario was described in a patient with Gitelman syndrome who required 260 mmol of K^+ per day. He developed an episode of acute renal failure due to volume depletion caused by gastroenteritis in the setting of concomitant administration of a nonsteroidal antiinflammatory drug (105). After complaining of weakness, he was found to have a serum K^+ concentration of 10.4 mmol/L. Use of dietary K^+ supplements may become more commonplace given the evidence that diets enriched in K^+ may be associated with clinical benefit (4).

CELLULAR REDISTRIBUTION

Cellular redistribution is more important as a cause of hyperkalemia than

as a cause of hypokalemia. Tissue damage is probably the most important cause of hyperkalemia due to redistribution of K^+ out of cells. This can be due to rhabdomyolysis, trauma, hypothermia (during the rewarming phase), burns, massive intravascular coagulation, and tumor lysis (either spontaneous or following treatment) (106).

Drugs may cause hyperkalemia as a result of cellular redistribution. An example of this complication is the use of succinylcholine to induce a paralytic state (107). Under normal circumstances, acetylcholine receptors are concentrated within the neuromuscular junction. The depolarization of these receptors by succinylcholine leads to efflux of intracellular K^+ but the accumulation of K^+ is confined to the neuromuscular junction such that no change in plasma K^+ concentration occurs. Hyperkalemia can develop when this drug is given under conditions that cause upregulation and widespread distribution of acetylcholine receptors throughout the whole muscle membrane. In this setting succinylcholine-induced depolarization of receptors causes clinically significant amounts of K^+ to enter the extracellular fluid space. Risk factors for this complication include denervation, prolonged immobilization, chronic infection, and burn injury.

Thalidomide is increasingly being used in the treatment of multiple myeloma and has been implicated in the development of hyperkalemia (108). The mechanism by which this occurs is not known but cellular shift has been suggested. Reports of this complication have been confined to patients with CKD.

Malignant hyperthermia is a rare clinical syndrome that is manifested by muscle rigidity, tachycardia, increased CO_2 production, skin cyanosis and mottling, rhabdomyolysis, and hyperkalemia. The onset of the disorder is usually within 1 hour of the administration of general anesthesia with the most common triggers being halothane and succinylcholine. The syndrome is due to a mutation in the gene that encodes the skeletal muscle ryanodine receptor. This receptor is a calcium channel that when mutated allows excess amounts of calcium to exit the sarcoplasmic reticulum resulting in tetany and heat production. Mutational analysis is now available to identify individuals who are at risk of this syndrome (109).

Mineral acidosis but not organic acidosis can cause a cell shift in K^+ (110). Sevelamer can lead to the development of metabolic acidosis and hyperkalemia due to this mechanism. The drug is a nonabsorbed polymer with covalently linked amino groups, almost half of which consist of amine hydrochloride. Chloride is released in exchange for monovalent phosphate in the gastrointestinal tract, so that for each molecule of

phosphate bound there is liberation of one molecule of hydrochloric acid. The substitution of chloride with carbonate (sevelamer carbonate) has eliminated this complication.

Hyperkalemic periodic paralysis is most commonly associated with mutations in the sodium channel *SCN4A* gene. In contrast to familial hypokalemic periodic paralysis, patients with the hyperkalemic form are typically younger (<10 vs. 5–20 years) and have a greater frequency of attacks that tend to be of shorter duration (<24 vs. >24 hours). The attacks can be precipitated by fasting and K^+ administration.

Decreased Renal Excretion of Potassium

Decreased renal excretion of K^+ can be divided into one or more of three abnormalities: a primary decrease in distal delivery of salt and water, abnormal cortical collecting duct function, and a primary decrease in mineralocorticoid levels (62) (Table 5-1).

PRIMARY DECREASE IN DISTAL DELIVERY (RENAL FAILURE)

Acute decreases in GFR as occurs in acute renal failure may lead to marked decreases in distal delivery of salt and water, which may secondarily decrease distal K^+ secretion. When acute renal failure is oliguric, distal delivery of NaCl and volume are low and hyperkalemia is a frequent problem. When acute renal failure is non-oliguric, however, distal delivery is usually sufficient and hyperkalemia is unusual.

Table 5–1 Causes of Hyperkalemia

- Pseudohyperkalemia
- Cellular redistribution
  - Mineral acidosis
  - Cell shrinkage (hypertonicity)
  - Deficiency of insulin
  - *β*-Blockers
  - Hyperkalemic periodic paralysis
  - Cell injury
- Excess intake (very rare)
- Decreased renal excretion

- Decreased distal delivery of Na^+ (oliguric renal failure)
- Mineralocorticoid deficiency
- Defect of cortical collecting tubule

The association of hyperkalemia and CKD is more complicated than with acute renal failure. In addition to decreased GFR and a secondary decrease in distal delivery, there is nephron dropout and a smaller number of collecting ducts to secrete K^+ with CKD. However, this is counterbalanced by an adaptive process in which the remaining nephrons develop an increased ability to excrete K^+. Two other defenses against hyperkalemia include a more rapid shift of K^+ into cells in response to a K^+ load and a markedly increased rate of K^+ excretion in the colon. For all of these reasons hyperkalemia is unusual in CKD patients with a slow decline until the GFR falls to <10 mL/minute. The occurrence of hyperkalemia with a GFR of >10 mL/minute (unless there has been a sudden decline in GFR, i.e., acute on chronic kidney failure) should raise the question of decreased aldosterone levels or a specific lesion of the cortical collecting duct.

Aldosterone continues to play a role in regulating K^+ even in anephric patients through stimulatory effects on colonic secretion. In this regard, fludrocortisone has been used sporadically to better control the plasma K^+ concentration in hyperkalemic chronic dialysis patients. To test whether the drug is effective in this setting, 37 patients with a midweek predialysis serum K^+ concentration of 5.1 to 5.3 mEq/L were randomized to receive either fludrocortisone at 0.1 mg/day or no treatment for a 3-month period (111). While the drug was safe and well tolerated, no difference was found in serum K^+ concentration between the two groups.

PRIMARY DECREASE IN MINERALOCORTICOID ACTIVITY

Decreased mineralocorticoid activity can result from disturbances that originate at any point along the RAAS. Such disturbances can be the result of a disease state or be due to effects of various drugs (112). Diabetes is perhaps the most common disease state associated with hyporeninemic hypoaldosteronism (110). Risk factors for development of hyperkalemia are given in Table 5-2. Hyperkalemia most commonly develops when one of more of these drugs are administered in a setting where the renin–angiotensin–aldosterone system is already impaired (113).

Hyperkalemia that develops in association with use of angiotensin-converting-enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) is of particular concern since patients at the highest risk of this complication are oftentimes the same individuals expected to derive the greatest cardiovascular benefit. It is worth emphasizing that in patients with CKD the level of renal function should not be the sole determinant as to whether these drugs should be initiated or continued. In a randomized, double-blind study of 224 patients with a serum creatinine concentration of 3.1 to 5.0 mg/dL, the administration of 20 mg/day of benazepril reduced the composite end point of doubling of the serum creatinine concentration, end-stage renal disease, or death as compared to placebo (114). During the 3 years of the study, both groups were treated with conventional antihypertensive drugs so that blood pressure control was no different. Hyperkalemia (defined as a serum $K^+ > 6.0$ mmol/L) developed in six patients treated with benazepril and five receiving placebo. Of these 11 patients, only three had to be withdrawn from the study. The other eight patients were successfully treated with dietary modifications, diuretic therapy, and optimized acid–base balance. The study illustrates that withholding these drugs simply on the basis of renal function can potentially deprive many patients the cardiovascular benefit they would have otherwise received, particularly because numerous steps can be taken to minimize the risk of hyperkalemia.

Table 5–2 Risk Factors for Development of Hyperkalemia

- Chronic kidney disease: risk is inversely related to GFR and increases substantially below an eGFR of 30 mL/min
- Diabetes mellitus[a]
- Decompensated congestive heart failure
- Medications
  - Inhibition of renin release from juxtaglomerular cells
    - Nonsteroidal antiinflammatory drugs
    - Beta blockers
    - Calcineurin inhibitors: cyclosporin, tacrolimus
  - Inhibition of aldosterone release from the adrenal gland
    - Heparin
    - Ketoconazole
  - Mineralocorticoid receptor blockade
    - Spironolactone
    - Eplerenone
  - Blockade of epithelial sodium channel blocker in renal collecting duct

- Amiloride
- Triamterene
- Trimethoprim

- Potassium supplements, salt substitutes, certain herbs, and K^+-enriched foods in the setting of impaired renal excretion

ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; GFR, glomerular filtration rate.

[a]A spectrum of abnormalities in the renin–angiotensin–aldosterone system have been described in patients with diabetes mellitus to include hyporeninemic hypoaldosteronism as well as normal renin release but a diminished capacity to release aldosterone. Hypoaldosteronism combined with dysfunction of collecting ducts due to diabetic nephropathy and treatment with ACEI or ARB's make these patients at particularly high risk of hyperkalemia.

The use of drugs that interfere in the renin–angiotensin system in patients at increased risk for hyperkalemia requires close monitoring. If the patient is to be initiated on an ACEI or an ARB, it is best to begin with low doses. The serum K^+ should be checked within 1 week of starting the drug and then the drug may be titrated upward if the K^+ remains in the normal range. With each increase in dose, the serum K^+ should be remeasured at 1-week intervals. For increases in the serum K^+ concentration up to 5.5 mEq/L the clinician may reduce the dose, and in some cases the K^+ concentration will improve allowing the patient to remain on a lower dose of the renin–angiotensin blocker (Table 5-3).

In a retrospective cohort study conducted in 10 health maintenance organizations, the frequency of serum K^+ and creatinine monitoring was assessed in patients labeled as being treated with ACEIs or ARBs for at least 1 year (115). More than two-thirds of the 52,906 patients identified received laboratory monitoring. The likelihood of monitoring increased with advancing age, more frequent outpatient visits, recent hospitalizations, concomitant use of drugs such as potassium salts, diuretics, and digoxin, and comorbidities such as diabetes, congestive heart failure, and CKD. Nearly one-third of patients prescribed these drugs had no laboratory monitoring over a 1-year period.

Table 5–3 Approach to Patients at Risk of Hyperkalemia When Using Drugs That Interfere in the Renin–Angiotensin–Aldosterone System

- Accurately assess level of renal function to better define risk
- Discontinue drugs that interfere in renal K^+ secretion, inquire about herbal preparations, and discontinue nonsteroidal antiinflammatory drugs to include the selective cyclooxygenase 2 inhibitors
- Low-K^+ diet; inquire about K^+-containing salt substitutes
- Thiazide or loop diuretics (loop diuretics necessary when estimated GFR is <30 mL/min)
- Sodium bicarbonate to correct metabolic acidosis in chronic kidney disease patients
- Initiate therapy with low-dose ACEI or ARB
  - Measure K^+ 1 week after initiation of therapy or after increasing dose of drug
  - For increases in K^+ up to 5.5 mEq/L, decrease dose of drug, if taking some combination of ACEI, ARB, and aldosterone receptor blocker discontinue one and recheck potassium
  - The dose of spironolactone should not exceed 25 mg daily when used with an ACEI or ARB; this combination of drugs should be avoided with GFR <30 mL/min
  - For K^+ ≥5.6 mEq/L despite above steps, discontinue drugs
- Consider chronic use of K^+ binding agents: patiromer, zirconium cyclosilicate

ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; GFR, glomerular filtration rate.

The discovery of hyperkalemia during laboratory testing does not guarantee appropriate follow-up. In a retrospective observational cohort study of a large primary care practice, 109 instances of hyperkalemia (defined as K^+ > 5.8 mEq/L) were identified in 86 patients (116). While more than half of the patients were recalled to the clinic for retesting, 25% of the cases had no repeat testing until they were seen on routine follow-up visits or when they came to the clinic for unrelated issues.

The lack of appropriate follow-up is of particular concern since the electrocardiogram in a hyperkalemic subject can progress from normal to that of ventricular tachycardia and asystole precipitously (117). Most physicians are familiar with the findings of peaked T waves and sine-wave pattern that typify hyperkalemia. Profound bradycardia is a less well appreciated manifestation of hyperkalemia (118). Particular attention should be given to patients with underlying disturbances of cardiac conduction since even mild degrees of hyperkalemia may precipitate heart block. This complication can occur regardless of whether the conduction

disease is intrinsic to the heart or drug induced.

Distal Tubular Defect

Certain interstitial renal diseases can affect the distal nephron specifically and lead to hyperkalemia in the presence of only mild decreases in GFR and normal aldosterone levels. Amiloride and triamterene inhibit Na^+ absorption by the principal cell in the cortical collecting duct (CCD), which makes the luminal potential more positive and secondarily inhibits K^+ secretion. A similar effect occurs with trimethoprim and accounts for the development of hyperkalemia following the administration of the antibiotic trimethoprim-sulfamethoxazole (113). Spironolactone and eplerenone compete with aldosterone and thus block the mineralocorticoid effect.

Pseudohypoaldosteronism type II (Gordon's syndrome) is an autosomal dominant form of hypertension in which hyperkalemia and metabolic acidosis are key features. Thus, the preferred designation of this syndrome is "familial hyperkalemic hypertension" or FHH. Plasma concentrations of aldosterone are low despite the presence of hyperkalemia, which normally exerts a stimulatory effect on aldosterone release from the adrenal gland. Administration of NaCl worsens the hypertension but Na^+ given with a non-chloride anion such as sulfate or bicarbonate has a beneficial effect. The hypertension and hyperkalemia are particularly responsive to the administration of thiazide diuretics.

Mutations in the protein kinases, WNK4 and WNK1, are responsible for FHH (119). Wild-type WNK4 acts to reduce the surface expression of the thiazide-sensitive Na^+/Cl^- cotransporter likely through a lysosomal-mediated degradative pathway (120). The mutant protein (inactivating mutation of WNK4) loses this capability resulting in increased cotransporter activity accompanied by marked hyperplasia of the DCT (121). The wild-type protein also stimulates clathrin-dependent endocytosis of the ROMK channel in the renal collecting duct leading to decreased cell surface expression. The mutant protein enhances this removal, giving rise to decreased K^+ secretion and hyperkalemia.

WNK4 has also been shown to affect Cl^- permeability through the paracellular pathway. The mutated WNK4 protein causes an increase in paracellular Cl^- permeability by phosphorylating claudins, which are tight junction proteins involved in regulating paracellular ion transport. This

increase in permeability dissipates the lumen-negative charge normally generated by Na^+ reabsorption via the ENaC channel. The reduction in luminal electronegativity will decrease the driving force for K^+ secretion thus providing an additional mechanism by which the mutated protein can cause hyperkalemia. This reduction in luminal electronegativity also contributes to the development of metabolic acidosis due to the less favorable electrical gradient for H^+ secretion. In addition, hyperkalemia slows H^+ secretion by limiting buffer availability through its suppressive effect on ammoniagenesis.

Mutations in WNK1 can also give rise to the manifestations of FHH. Wild-type WNK1 normally exerts an inhibitory effect on WNK4. Mutations in WNK1 that give rise to pseudohypoaldosteronism type II are gain-of-function mutations that augment this inhibitory effect on WNK4 activity. As a result, Na^+/Cl^- cotransport activity is increased and there is increased removal of ROMK from the apical membrane. WNK1 can also cause salt retention by increasing the activity of ENaC through a stimulatory effect on SGK1. In addition, increased activity of WNK1 enhances paracellular Cl^- permeability in a similar manner as disease-causing mutations in WNK4 (122). This increase in Cl^- permeability may be related to WNK1-mediated phosphorylation of claudin-4. The observation that hypertension in patients with the WNK1 mutation is less sensitive to the effects of thiazide diuretics suggests these non-Na^+/Cl^- cotransporter mechanisms of salt retention are quantitatively more important in causing volume expansion in this setting. While less effective in treating hypertension, thiazide diuretics remain effective in correcting hyperkalemia despite the augmented removal of ROMK. As with the inactivating mutations in WNK4, flow-mediated K^+ secretion via maxi-K channels likely accounts for this effect.

One additional difference in the clinical manifestations resulting from mutations in WNK4 and WNK1 relates to urinary calcium excretion. Increased Na^+/Cl^- cotransporter activity is normally associated with hypercalciuria while inhibition of the cotransporter decreases urinary calcium excretion. This later effect explains the hypocalciuric effect of thiazide diuretics. Patients with the WNK4 mutation are hypercalciuric and show a heightened sensitivity to the hypocalciuric effects of thiazide diuretics when compared to normal subjects (123). These findings are consistent with constitutive activation of the Na^+/Cl^- cotransporter as the major cause of salt retention in patients with the WNK4 mutation. By contrast, hypercalciuria is not a feature in patients with the WNK1

mutation suggesting increased ENaC activity and paracellular Cl^- permeability play a more important role in mediating salt retention in these patients as compared to increased Na^+/Cl^- cotransporter activity.

Pseudohypoaldosteronism type I a disorder characterized by mineralocorticoid resistance that typically presents in the newborn. Clinical findings include hyperkalemia, metabolic acidosis, and a tendency toward volume depletion due to renal salt wasting. There are two modes of inheritance that give rise to slightly different characteristics. In the autosomal recessive form of the disease, the defect is localized to homozygous mutations in the three subunits of the epithelial sodium channel. This form of the disease tends to be more severe and requires lifelong therapy with salt to prevent recurrent life-threatening volume depletion. Extrarenal manifestations include frequent respiratory tract infections due to the presence of dysfunctional channels in the lung. Cutaneous lesions can also develop as a result of the chronic irritative effect of high salt concentrations in sweat (124).

The autosomal dominant form of the disease is due to mutations in the mineralocorticoid receptor that result in mineralocorticoid resistance. The clinical manifestations are limited to the kidney and tend to resolve with time such that therapy with potassium binding resins and salt supplementation can eventually be discontinued. The maintenance of normal volume homeostasis and electrolyte values occurs at the expense of a persistent increase in circulating aldosterone levels in adults with the disorder (125). Depending on the mutation, several types of disturbances on receptor function have been described. These include a marked decrease in affinity to the total absence of binding of aldosterone to the receptor (126). Other types of mutations lead to a failure of initiating transcription or prevent the receptor from translocating into the nucleus. In unrelated families with the disease, loss-of-function mutations have been found to cluster within specific codons, suggesting there are mutational hot spots within the mineralocorticoid receptor gene (127).

Clinical Manifestation of Hyperkalemia

All of the clinically important manifestations of hyperkalemia occur in excitable tissue. Neuromuscular manifestations include paresthesias and fasciculations in the arms and legs. As the serum K^+ continues to rise, an ascending paralysis with eventual flaccid quadriplegia supervenes.

Classically, trunk, head, and respiratory muscles are spared, but rarely respiratory failure can occur.

The depolarizing effect of hyperkalemia on the heart is manifested by changes observable in the electrocardiogram (ECG). The progressive changes in hyperkalemia are classically listed as peaking of T waves, ST-segment depression, widening of the PR interval, widening of the QRS interval, loss of the P wave, and development of a sine-wave pattern. The appearance of a sine-wave pattern is ominous and is a harbinger of impending ventricular fibrillation and asystole.

Hyperkalemia can also be associated with a number of less common patterns on the ECG. Brugada syndrome is a genetic disease associated with sudden cardiac death due to mutations in a cardiac Na^+ channel. ECG changes are characterized by a right-bundle branch block pattern and right precordial ST-segment elevations. A similar pattern has been reported in patients with hyperkalemia. However, the hyperkalemic Brugada pattern differs from the genetic disorder in that P waves are often absent, abnormal axis deviation is present, and the QRS complex is wider (128). Hyperkalemia can also give rise to ECG changes typical of cardiac ischemia (129).

The correlation of ECG changes and serum K^+ concentration depends on the rapidity of the onset of hyperkalemia. Generally, with acute onset of hyperkalemia, ECG changes appear at a serum K^+ of 6 to 7 mEq/L. However, with chronic hyperkalemia, the ECG may remain normal up to a concentration of 8 to 9 mEq/L. Despite these generalities, clinical studies show a poor correlation between serum K^+ concentration and cardiac manifestations. In a retrospective review, only 16 of 90 cases met strict criteria for ECG changes reflective of hyperkalemia (defined as new peaked and symmetric T waves that resolved on follow-up) (130). In 13 of these cases, the cardiologist read the ECG as showing no T-wave changes. Strict ECG changes were only noted in 1 of 14 hyperkalemic patients who manifested arrhythmias or cardiac arrest, which calls into question the prognostic use of the ECG in this setting. Given the poor sensitivity and specificity of the ECG, the authors stress that the clinical scenario and serial measurements of K^+ are the preferred tools to guide the management of patients with hyperkalemia.

The treatment of acute hyperkalemia depends on the degree of the increase in the plasma potassium concentration and whether there are ECG changes or neuromuscular symptoms. A plasma potassium concentration above 7.5 mEq/L, severe muscle weakness, or marked ECG changes are

potentially life threatening and require immediate and emergent treatment.

Calcium antagonizes the potassium-induced decrease in membrane excitability, restoring membrane excitability toward normal. Hyperkalemia causes sustained subthreshold depolarization, which inactivates sodium channels, rendering the membrane progressively less excitable. The toxicity of hyperkalemia is worsened in patients with coexistent hypocalcemia. Elevation of plasma calcium concentration in hyperkalemic patients normalizes the difference between the resting and threshold potentials and restores sodium channel activity. The protective effect of Ca^{2+} administration is quite rapid and should be used only in patients in whom the P wave is absent or the QRS is widened. The usual dose is 1 ampule (10 mL) of a 10% calcium gluconate solution infused over 2 to 3 minutes under ECG monitoring. This dose can be repeated after 5 minutes if the ECG changes persist.

Insulin lowers the plasma potassium concentration by driving potassium into cells. Insulin is administered as 10 units of regular insulin with 30 to 50 g of glucose to prevent hypoglycemia. This regimen lowers the plasma potassium concentration by 0.5 to 1.5 mEq/L. The effect of insulin is evident within 30 to 60 minutes and may last for 2 to 4 hours. An ongoing glucose infusion after the initial insulin-glucose treatment may be necessary to prevent late hypoglycemia.

Raising the extracellular pH with $NaHCO_3$ drives potassium into the cells when given to patients with metabolic acidosis. The usual dose is 44 to 50 mEq of $NaHCO_3$ infused over 5 minutes. The effect begins within 30 to 60 minutes and may persist for 2 to 4 hours. In the absence of acidosis, a beneficial effect of $NaHCO_3$ is more difficult to demonstrate. Given this lack of efficacy and potential to cause extracellular fluid volume expansion, bicarbonate administration should only be considered for the hyperkalemic acidemic patient who already has received calcium, insulin, and glucose, and perhaps an adrenergic agent.

Activation of β_2-adrenergic receptors drives potassium into the cells. Albuterol, 10 to 20 mg by nebulizer, can lower the plasma potassium concentration by 0.5 to 1.5 mEq/L within 30 to 60 minutes. The effects of calcium, insulin, $NaHCO_3$, and β_2-agonists are only transient. For the long-term achievement of normokalemia, these acute treatment modalities need to be followed by managements that remove the excess potassium from the body. These treatments include diuretics, cation-exchange resins, and dialysis.

Pharmacological management of hyperkalemia has relied for over 50

years on chronic use of sodium polystyrene sulfonate (Kayexalate) that binds K^+ in the gastrointestinal tract; however, this is poorly tolerated and has been linked to gastrointestinal toxicity. Moreover, long-term administration is linked to serious side effects such as rare cases of intestinal necrosis resulting in a black box warning by the Food and Drug Administration. Recently, new oral compounds, patiromer and sodium zirconium cyclosilicate (ZS-9), which are K^+ binding drugs have been shown to be effective in preventing the development of hyperkalemia. Patiromer is approved for clinical use and ZS-9 is pending approval. Both agents exhibit good tolerability and are not associated with serious adverse effects. Recently, clinical trials demonstrated these compounds lower the risk of incident hyperkalemia associated with RAAS blockade in people with diabetes, heart failure, and/or who have CKD. Patiromer (Veltassa) is a nonabsorbed polymer that binds K^+ in the gastrointestinal tract, predominately in the colon. Patiromer effectively decreases serum K^+ concentrations in high-risk patients on RAAS blockers, including those with heart failure, CKD, and diabetic nephropathy (131). In a study of over 300 patients with diabetic nephropathy with either mild to moderate hyperkalemia, the drug lowered serum K^+ concentration in a dose-dependent manner with the greatest reduction in those with higher starting values. The drug remained effective in controlling plasma K^+ concentration over a 44-week maintenance phase despite ongoing administration of RAAS inhibitors (132). The drug was well tolerated with the main adverse events being constipation (infrequent and self-limiting) and hypomagnesemia, which required magnesium replacement in a small number of subjects.

Sodium zirconium cyclosilicate is a nonabsorbed microporous compound that binds K^+ throughout the gastrointestinal tract. The pore size renders it highly selective for the K^+ ion as compared to calcium or magnesium ions. Like patiromer, this drug has also been effective in lowering plasma K^+ concentration in a dose-dependent manner with the greater reductions in those with the highest levels (133,134). However, despite being well tolerated, there are reports of edema at higher doses. Unlike patiromer, there is limited evidence suggesting that the rapidity of the potassium-lowering effect that is faster for sodium zirconium cyclosilicate than for patiromer renders the former agent to be a possible adjunct agent for the treatment of acute, as well as chronic hyperkalemia.

Dialysis is needed to remove the excess potassium load in patients with severe hyperkalemia, especially in the presence of advanced renal failure

or when accompanied by a hypercatabolic state or severe tissue necrosis. Acute hemodialysis is more effective than peritoneal dialysis in this setting.

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CHAPTER 6

Disorders of Calcium, Phosphorus, Vitamin D, and Parathyroid Hormone Activity

Mordecai M. Popovtzer

Serum Calcium Concentration

The calcium ion is essential to any physiologic phenomena, including preservation of the integrity of cellular membranes, neuromuscular activity, regulation of endocrine and exocrine secretory activities, blood coagulation, activation of the complement system, and bone metabolism.

Total Serum Calcium Concentration

The normal range for total serum calcium must be established for each laboratory and varies according to the method used. Total serum calcium is divisible into protein-bound and ultrafiltrable (diffusible) calcium (Fig. 6-1).

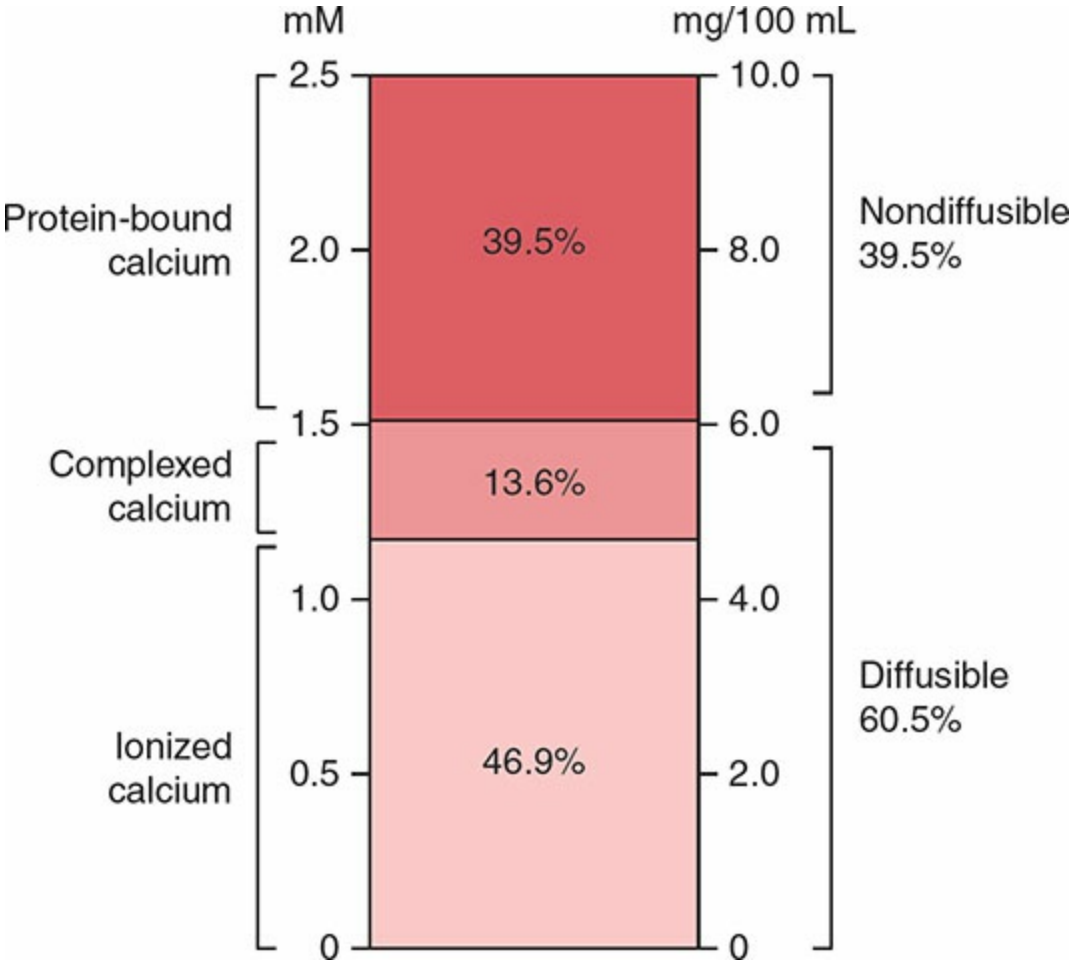


Figure 6–1 Calcium fractions in the serum. (Republished with permission of American Society for Clinical Investigation from Moore EW. Ionized calcium in normal serum, ultrafiltrates and whole blood determined by ion-exchange electrode. *J Clin Invest.* 1970;49:318; permission conveyed through Copyright Clearance Center, Inc.)

PROTEIN-BOUND CALCIUM

Approximately 40% of total calcium is bound to serum proteins, and 80% to 90% of this calcium is bound to albumin. Variations in serum protein alter proportionately the concentration of the protein-bound and total serum calcium. An increase in serum albumin concentration of 1 g/dL increases protein-bound calcium by 0.8 mg/dL, whereas an increase of 1 g/dL of globulin increases protein-bound calcium by 0.16 mg/dL. Thus, it is obvious that changes in total serum calcium concentration cannot be used for the assessment of the effect on bound calcium concentration unless the changes in albumin and globulin concentrations also are determined. Marked changes in serum sodium concentration also affect the protein binding of calcium.

Hyponatremia increases, whereas hypernatremia decreases protein-

bound calcium. Changes in pH also affect protein-bound calcium, and an increase or decrease of 0.1 pH increases or decreases protein-bound calcium by 0.12 mg/dL. Respectively, in vitro, freezing and thawing serum samples may decrease the binding of calcium as well.

ULTRAFILTRABLE (DIFFUSIBLE) CALCIUM

Serum ultrafiltrable calcium is obtained by applying pressure on serum against a semipermeable membrane. Thus, serum water is forced across the membrane, and the ultrafiltrate is analyzed for calcium concentration and then corrected for total serum solids. The samples must be handled anaerobically, because changes in pH may affect calcium binding. Under normal conditions, ultrafiltrable calcium constitutes 55% to 60% of the total serum calcium.

FREE (IONIZED) CALCIUM

The biologically active component of diffusible calcium is ionized calcium. Flow-through and static ion exchange electrodes, which function similarly to conventional pH electrodes, are used. Serum-ionized calcium concentration in normal subjects ranges from 4.0 to 4.9 mg/dL, or 47% of total serum calcium. The samples have to be handled anaerobically because changes in pH alter the concentration of ionized calcium. Determinations are best made on freshly separated serum, because heparin creates complexes with calcium, and the presence of fibrin may interfere with the structural integrity of the porous membrane used in the procedure. Storage of serum in oil does not prevent changes in pH, because carbon dioxide dissolves readily in oil. An increase in serum pH of 0.l unit may cause a decrease in ionized calcium of 0.16 mg/dL (1,2). As with ultrafiltrable calcium, freezing and thawing of serum may alter the level of ionized calcium.

COMPLEXED CALCIUM

The nonionized portion of diffusible or ultrafiltrable calcium is called complex calcium. The calcium complexes are formed with bicarbonate, phosphate, and acetate. The amount of complexed calcium is measured indirectly by subtracting the ionized calcium (47%) from the ultrafiltrable calcium (60%) and thus equals about 13% of total serum calcium. The complexed calcium has been found to be increased twofold in patients

with uremia.

CYTOSOLIC CALCIUM

Cytosolic calcium can be measured by loading the tested cells with a fluorescent probe such as indo-1-acetoxymethyl ester and exciting the cells at 350 nM. The ratio of fluorescence emission at 410 nM to that at 490 nM is used as an index of free intracellular calcium. The normal concentration of cytosolic calcium is 100 nM/L, which is 10,000-fold lower than the concentration of extracellular calcium. The very steep gradient is maintained by an energy-driven calcium pump, known as the plasma membrane Ca^{2+} ATPase (PMCA). In certain types of cells, a Na^{+}/Ca^{2+} exchanger, energized by a Na^{+}-gradient, helps drive cytosolic calcium into the extracellular space. Part of cellular calcium is sequestered in intracellular organelles, including endoplasmic reticulum, sarcoplasmic reticulum (SR) in muscle cells and in mitochondria. The calcium-dependent intracellular signaling generally requires a 10-fold increase in cytosolic calcium. With each heartbeat, the cytosolic calcium concentration in cardiac myocytes is elevated 10-fold, from a resting level of 100 to 1,000 nM. Likewise, in other signaling events such as T-cell activation, which triggers the transcription of interleukin-2, a 10-fold increase in cytosolic calcium serves as the signal for the response. Elevation of cytosolic calcium is mediated by activation of calcium channels, which allows passive calcium flux down its electrochemical gradient.

Calcium plays an important role in cardiac coupling of excitation and contraction. The lasting phase of cardiac action potential is maintained by L-type calcium channels, whereby extracellular calcium influx into the intracellular space activates calcium-sensitive channels of SR. Relatively small amounts of calcium entering the cell via the L-type channel activate a small number of SR calcium channels. This leads to calcium release from SR and a marked increase in intracellular calcium concentration, termed “calcium-induced calcium release.” Calcium removal from the cytoplasm occurs via active transport back to SR by calcium pump, Ca^{2+} ATPase. A smaller amount, the same amount that entered the cell through L-type calcium channel, is extruded into the extracellular space via the sodium–calcium exchanger (NCX). Glycosides, the drugs known for positive inotropic effect, act by inhibiting the sodium pump (Na^{+}/K^{+} ATPase), increase intracellular sodium load in the myocytes. Subsequent activation of NCX leads to sodium extrusion and calcium into the myocyte

cytosol. Glycosides represented the only effective therapy for congestive heart failure for a long time (3).

Serum Phosphorus Concentration

Serum phosphorus occurs in two forms, organic and inorganic. Organic phosphorus is composed entirely of phospholipids bound to proteins. The inorganic fraction is the principal circulating form of phosphorus and is routinely assayed for clinical uses. About 90% of inorganic phosphorus is ultrafiltrable. About 53% of the ultrafiltrable inorganic phosphorus in serum is dissociated with a 1:4 ratio of $H_2PO_4^-$ to HPO_4^{2-}; the remainder of ultrafiltrable phosphate is in the form of salts of sodium, calcium, and magnesium. During marked hyperphosphatemia (8–10 mg/dL of serum), a significant portion of phosphate forms colloidal complexes with calcium that are rapidly removed from the circulation.

Studies propose that an increase in serum phosphorus leads to a reciprocal fall in serum calcium, so that the product of both ions remains constant. The assumption was the solubility equilibrium exists between the bone and the extracellular fluid (ECF). However, this appears to be an oversimplification of a more complex equilibrium. An inverse relationship between serum calcium and phosphorus is present only under extreme changes in serum phosphorus; for example, a decrease in serum calcium occurs following an acute rise in serum phosphorus. By contract, this relationship does not hold for acute changes in serum calcium, because a rapid increase in calcium leads to a rise rather than a fall in serum phosphorus before any changes in urinary phosphorus occur (2). This effect may be caused by the release of phosphorus from cells. Serum phosphorus displays circadian variations. Serum phosphorus levels reach a nadir early in the morning, with an increase to plateau at 4 p.m., followed by a further increase to a peak at 1 to 3 a.m.

Serum phosphorus concentration is also influenced by age. In adults, the normal concentration ranges from 2.5 to 4.0 mg/dL, whereas in children it ranges from 4 to 6 mg/dL. The level of alkaline phosphatase in children is higher than in adults. These age differences are probably related to different rates of skeletal growth. Serum phosphorus decreases during hyperventilation and alkalosis and increases during acidosis. Serum phosphorus also varies directly with its content in the diet. Administration of glucose causes a fall in serum phosphate because of the flux of

phosphate into cells with the phosphorylation of glucose. The administration of insulin and epinephrine also reduces serum phosphorus concentration. Hypophosphatemia occurring in sepsis and acute myocardial infarction may result from the release of epinephrine into the circulation.

A recent large population study showed that serum phosphorus is only weakly related to dietary phosphorus intake and/or phosphorus-rich foods, suggesting that other factors determining serum phosphorus concentration remain to be defined (4). This finding may bear on another earlier population study that showed that higher serum phosphorus levels, even within the normal range, had been associated with increased risk of cardiovascular events in patients with normal kidney function (5). The clinical relevance of these and other population-wide studies is uncertain.

Calcium and Phosphorus Balance

Total body calcium ranges from 1.0 to 1.5 kg and that of phosphorus from 0.5 to 0.8 kg. Ninety-nine percent of total calcium and 85% of total phosphorus are stored in the skeleton. Only 1% of both are in the ECF, and the remainder is intracellular.

DIETARY CALCIUM AND PHOSPHORUS

Dietary calcium and phosphorus intake varies considerably. In general, balanced diets provide from 800 to 1,200 mg of calcium and from 800 to 1,500 mg of phosphorus per day. The minimum daily requirement of calcium is 400 to 500 mg, and an intake below this amount may cause a negative calcium balance. Dietary calcium can be reduced to about 200 mg by the exclusion of dairy products (6), and boiling of vegetable causes a loss of 25% of their calcium content. It has become common to enrich bread with powdered milk to increase the amount of calcium in the diet. Drinking water is also a source of calcium; “soft” water has 1 to 3 mg/dL of calcium, and “hard” water has 3 to 10 mg/dL. Human diets, almost without exception, contain more phosphorus than calcium, because phosphorus is present in almost all foodstuffs. The amount of calcium and phosphorus in various foods is shown in Table 6-1.

INTESTINAL ABSORPTION OF CALCIUM

Calcium is absorbed along the small intestine, more in the duodenum and proximal jejunum than in the ileum (6–8). The absorption of calcium is completed within 4 hours after its oral intake (8,9). Calcium absorption in the gastrointestinal (GI) tract occurs via two transport processes (10–13). Transcellular calcium absorption, which is saturable and physiologically regulated, follows three steps: (a) luminal entry into mucosal cells through apical calcium channels; (b) binding to a protein carrier, calbindin 9k, and transfer to the serosal side; and (c) extrusion from the cell by an active process at the basolateral side by Ca^{2+} ATPase (calcium pump) and also most likely, by NCX. Increasing body demands for calcium activate maximally the transcellular transportation. Paracellular calcium absorption is nonsaturable and is driven by concentration gradients between luminal and serosal spaces. Thus, the rate of absorption depends primarily on calcium concentration in the lumen. This pathway of absorption predominates in the distal small bowel. The paracellular absorption route traverses the apical tight junctions of the mucosal cells; therefore, changes in permeability in these sites also may affect the rate of transport. In contrast to the paracellular pathway, the transcellular route represents an actively controlled mechanism of calcium reabsorption. Identification of epithelial calcium channels TRPV6 and, to a lesser extent, TRPV5 advanced our understanding of transcellular calcium reabsorption (14). It appears that TRPV5/6 is regulated by vitamin D. ECaC was first cloned from a rabbit kidney cortex. In addition to the kidney, TRPV messenger ribonucleic acid (mRNA) is expressed in the small intestine as well.

Immunohistochemical staining located the channel protein in the apical membrane of renal epithelial cells and the brush-border membrane of duodenal and jejunal villi. TRPV mRNA and protein abundance are decreased in states of vitamin D deficiency and increased with vitamin D repletion. TRPV is the rate-limiting factor in active calcium absorption by regulating the apical entry of calcium into the epithelial cells. The TRPV family consists of two homologous species; TRPV refers mainly to the renal channel, whereas ECaC2 is mainly the intestinal channel. Genomic cloning showed that TRPV5 and TRPV6 are products of distinct genes; both are juxtaposed on chromosome 7q35, suggesting evolutionary gene duplication. TRPV6 has been cloned from rat, mouse, and human intestines.

The absorption of calcium becomes more efficient with low calcium intake, thus ensuring that adequate amounts of calcium are delivered to the body. This process of adjustment to low calcium intake, which is not entirely understood, has been termed “adaptation.” Younger persons

exhibit this phenomenon of adaptation better than older individuals. The absorption of calcium also increases in direct proportion to the requirements; for example, calcium absorption increases during pregnancy and depletion of total body calcium.

Table 6–1 Calcium and Phosphorus Content in Different Foods

Food	Calcium (mg/100 g)	Phosphorus (mg/100 g)
Cow's milk	120	100
Hard American cheese	697	771
Cottage cheese	100	110
Eggs	54	205
Meat	13	200

Oral calcium may be complexed, chelated, or precipitated in the GI tract by a variety of substances that render it unavailable for absorption. These substances include phytate, oxalate, and citrate. Certain drugs, including colchicine, fluoride, theophylline, and glucocorticoids, also interfere with calcium absorption. Rapid motility or shortening of the length of the GI tract may diminish the absorption of calcium as well. Decreased calcium absorption has been observed with protein depletion both in human subjects and in rats. A deficiency of the specific calcium-binding protein in the intestinal mucosal cells has been proposed as the mechanism accounting for this failure of calcium transport.

In the absence of oral intake, calcium continues to be excreted in the feces and a negative calcium balance ensues. Thus, it is apparent that some of the fecal calcium is derived from intestinal secretion. Using an intravenous tracer method, the daily calcium secretion has been estimated to be on the order of 150 mg/day. This amount does not change during an intravenous load of calcium.

Net calcium absorption (dietary calcium minus fecal calcium) can be determined by maintaining the patient on a constant diet and collecting stools. This balance method is time consuming, because it requires an equilibration period of several days followed by a collection period of several days. The results may be expressed in absolute values, where the net calcium absorption is the difference between calcium intake and calcium fecal excretion. Alternatively, the results can be expressed as

fractional calcium absorption, as shown in the following formula:

$$\text{Fractional calcium absorption} = \frac{\text{dietary calcium} - \text{fecal calcium}}{\text{dietary calcium} \times 100} \quad (6.1)$$

INTESTINAL ABSORPTION OF PHOSPHORUS

About 50% to 65% of dietary phosphorus is absorbed, mostly in the jejunum. Evidence from in vitro studies indicated that phosphorus absorption is an active process. The active process is sodium coupled and saturable.

Phosphate is transported across the mucosal brush-border membrane against an electrochemical gradient. This active transport is sodium dependent and is driven by a sodium gradient generated and maintained by the activity of Na^+/K^+ ATPase at the basolateral membrane. A sodium-phosphate (NaPi) cotransporter has been cloned from the mouse small intestine and designated as a type IIb (NaPiIIb) cotransporter in analogy to a type IIa (NaPiIIa) cotransporter cloned from the kidney (15). Type IIb is located in human chromosome 4, whereas type IIa (NaPiIIa) is located in human chromosome 5. NaPiIIb protein was localized in the intestinal brush-border membrane vesicles by Western analysis. The type IIb (NaPiIIb) cotransporter was characterized by functional studies in complementary RNA (cRNA)-injected oocysts and shown to have the features of intestinal NaPi transport. Type IIb-mediated NaPi cotransport is enhanced by more acidic pH as opposed to the renal type IIa transporter, which is stimulated by more alkaline pH. It has been shown that $1,25(OH)_2D$ increases phosphorus transport by stimulating NaPiIIb cotransporter. Experimental studies in rodents demonstrated age-dependent response to $1,25(OH)_2D$. In suckling animals, vitamin D increased NaPiIIb gene expression and protein abundance, whereas in adult rodents $1,25(OH)_2D$ increased protein abundance without changes in gene expression of the cotransporters in the small intestine (16). Similarly, low-phosphorus diet-stimulated intestinal phosphorus absorption is associated with NaPiIIb protein abundance without changes in gene expression. This response is independent of vitamin D (17). Nicotinamide-induced inhibition of intestinal phosphorus absorption is associated with a decrease in NaPiIIb protein abundance in brush-border membrane vesicles.

There is, however, a linear correlation between phosphorus intake and net absorption. This reflects the passive paracellular pathway of transport, which is determined by concentration gradients of phosphorus across the

intestinal mucosa. In contrast to findings in animals, high phosphate intake in humans does not seem to cause a decrease in calcium absorption. Rather, the presence of phosphate in the diet is necessary for calcium absorption. Phosphate absorption may be decreased by a high calcium intake or ingestion of aluminum hydroxide antacids, which bind phosphorus in the bowel, thereby inhibiting its absorption. Similarly, sevelamer (Renagel), lanthanum, and sucroferric oxyhydroxide can reduce intestinal phosphate absorption.

URINARY EXCRETION OF CALCIUM

The urinary excretion of calcium varies considerably in normal subjects, but the oral intake only modestly affects the daily urinary excretion of calcium. The upper normal range of calcium excretion per day has been estimated to be <300 mg for men and <250 mg for women, or 4 mg/kg body weight. Unlike the response to a low-sodium diet, institution of a diet very low in calcium does not lead immediately to a substantial reduction in urinary calcium. However, in clinical states of protracted calcium depletion, such as in patients with intestinal malabsorption and osteomalacia, urinary excretion of calcium may be reduced to 50 mg/day or less.

Only ultrafiltrable calcium crosses the glomerular capillary walls and is then partially reabsorbed by the tubular epithelial cells. In adults, 97% to 99% of filtered calcium is reabsorbed. The tubules reabsorb ionized calcium more easily than complexed calcium, which accounts for the fact that the proportion of ionized calcium in the urine is only 20% of the total, the remainder being complexed calcium. The calcium complexes contain many anions such as citrate, sulfate, phosphate, and gluconate. Citrates in the urine bind calcium most powerfully. Sixty percent of calcium is chelated with citrate at a neutral pH in 1 L of urine containing 100 mg of calcium and 480 mg of citrate; this fraction falls to 40% at a pH of 5.0.

Urinary excretion of calcium is influenced by oral intake and urinary excretion of sodium. Thus, any attempt to assess urinary calcium excretion must take into account the oral intake and excretion of sodium. It has also been found that factors that affect the renal excretion of sodium, such as ECF volume expansion, similarly alter the renal excretion of calcium. Chronic expansion of ECF volume with mineralocorticoid hormone increases the urinary excretion of calcium as well.

It has been estimated that 50% to 70% of filtered calcium is reabsorbed in the proximal nephron, 30% to 40% is reabsorbed between the end of the

accessible part of the proximal tubule and the distal tubule (DT), and the remaining 10% is reabsorbed in the distal nephron (13,18). Micropuncture studies have demonstrated that sodium and calcium exhibit very similar absorptive characteristics in the proximal tubule. In the thick ascending limb (TAL) of the loop Henle, the absorption of both ions follows the same direction. Lumen-positive voltage is the driving force for calcium reabsorption in this segment. Furosemide abolishes the transepithelial potential; therefore, it reduces calcium absorption in parallel with reduced sodium reabsorption. Parathyroid hormone (PTH) reduces urinary excretion of calcium but augments urinary excretion of sodium.

Recent studies have provided more detailed insight into the tubular mechanisms underlying calcium transport along various nephron segments. The main fraction of filtered calcium is reabsorbed via paracellular passive flux that is driven by an electrochemical gradient in the proximal tubule and in the TAL, accounting for 80% to 90% of total filtered calcium. Epithelia permit selective and regulated flux from apical to basolateral surfaces by paracellular flux between cells or transcellular passage through cells. Tight junctions constitute the route for paracellular conductance in TAL for divalent cations, whereas the epithelial calcium channel TRPV5, in the distal convoluted tubule (DCT) and connecting tubule (CNT) constitute the apical entry mechanism of active transcellular calcium transport. Two conditions have to be met in TAL for paracellular reabsorption of divalent cations—the transepithelial voltage must be oriented lumen positive, and the paracellular route must allow divalent cation conductance. In this regard, alterations in transepithelial NaCl reabsorption are a determinant of divalent cation reabsorption by means of changes in transepithelial voltage generation. The observations that humoral factors (e.g., PTH) and basolateral concentration changes of divalent ions without changes in transepithelial voltage alter calcium reabsorption suggest a selective effect on paracellular permeability with specificity to divalent cations.

Claudins are members of tight-junction membrane proteins that act both as paracellular pores and as barriers that express selectivity to ions. Claudin-16 (originally paracellin 1) and claudin 19 are expressed in the thick ascending loop of Henle (TALH). Both facilitate paracellular absorption of divalent ions. In vitro and in vivo studies have shown that both claudin-16 and claudin-19 play a role in paracellular reabsorption of calcium and magnesium. The lumen-positive potential is proposed to be the driving force for divalent ion, Mg^{2+} and Ca^{2+}, reabsorption. In this regard, mutations of claudin-16 and claudin-19 genes cause autosomal

recessive familial hypomagnesemia, hypercalciuria, and nephrocalcinosis (FHHNC).

Recently, a new claudin, claudin-14, was identified in TALH; it is associated with kidney stone disease. The underlying mechanism for hypercalciuria and stone formation is inhibition of claudin-16 by claudin-14. Sequence variants in claudin-14 gene are associated with kidney stones and low bone density.

Claudin-14 is upregulated by activation of calcium sensing receptor (CaSR). Upregulation of CaSR as in the case of hypercalcemia leads to activation of claudin-14 and inhibition of claudin-16, leading to hypercalciuria resembling the phenotype of FHHNC (19–22).

Extracellular CaSR plays a key role in calcium and magnesium reabsorption in TAL. Extracellular calcium and other cations (e.g., Mg^{2+}) activate mechanism(s) that control their paracellular tubular transport by acting on the basolateral CaSR that recognizes those cations as their extracellular ligands. CaSR is a G protein-coupled receptor that leads to a transient rise in intracellular calcium by activation of phospholipase C, hydrolysis of phosphatidylinositol 4,5- to biphosphate, and increased by the formation of inositol triphosphate and diacylglycerol. CaSR is expressed by the cells of the TAL and plays a role in the normal regulation of calcium absorption in the nephron segment. Thus, normal or high calcium concentration is likely to be “sensed” by CaSR activating a signal transduction cascade and leading to reduced paracellular calcium reabsorption. Conversely, low calcium concentration (i.e., hypocalcemia) would fail to activate the signal transduction pathway, thus leading to abnormally avid calcium reabsorption and hypocalciuria. The latter also explains the hypocalciuria observed in patients with familial hypocalciuric hypercalcemia (FHH), in whom the gene that encodes the CaSR has undergone an inactivating mutation, leading to a defective receptor (23). Theoretically, the CaSR-initiated signaling could regulate calcium absorption by three putative mechanisms: (1) altering the permeability to calcium of the paracellular tight junction pathway, (2) changing the apical electrolyte transport that generated the TAL luminal electropositivity that is the driving force for calcium absorption, or (3) both.

Polymorphism with the R990G allele, which results in gain of function of CaSR, has been reported. This polymorphism has been associated with increased susceptibility to hypocalciuria and renal stone formation (24).

The fine-tuning of tubular calcium reabsorption, which is crucial for the maintenance of calcium balance, is regulated by active transcellular calcium transport in distal nephron segments. The calcium channel TRPV5

plays an important role in the process (14).

Immunohistochemical analysis demonstrated the presence of TRPV5 in the apical membrane of late DCT2 and CNT of the kidney cortex. TRPV5 is believed to constitute the rate-limiting mechanism, the first step in active calcium reabsorption. Following apical entry into the cytosol, calcium is bound to a carrier protein (calbindin D_{28K}) that translocates the cation to the transporters residing in the basolateral cell surface, the NCX and ATP-dependent calcium pump, the PMCA, which extrude the calcium to the extracellular basolateral space. The latter can be inhibited by calcium via the CaSR that resides at the basolateral membrane of DCT.

PTH and 1,25-dihydroxycolecalciferol ($1,25[OH]_2$ D3) are the major regulators of TRPV5 in the distal nephron. Parathyroidectomy in rats leads to a fall in TRPV5 expression, as well as diminished calbindin D_{28K} and NCX. PTH supplementation restores the expression of TRPV5. $1,25(OH)_2D_3$ increases the expression of TRPV5, calbindin D_{28K}, PMCA1b, and NCX, thus harmonizing enhanced calcium absorption.

Recent observations suggest that Klotho, the antiaging hormone, upregulates distal calcium reabsorption by two putative mechanisms. The Klotho gene encodes a single-pass transmembrane protein with a sequence similar to the β-glucosidase enzyme. The extracellular domain of Klotho, pKlothorotein, is shed and present in the circulation and tubular lumen, potentially functioning as a human factor. First, in response to low extracellular calcium levels, Klotho binds to Na^+/K^+ ATPase and translocates it to the plasma membrane. The increased sodium gradient generated by Na^+/K^+ ATPase drives the transepithelial transport of calcium by activating the basolateral NCX. Second, Klotho in the urine increases TRPV5 abundance on the luminal surface by hydrolyzing the *N*-linked extracellular sugar residues of TRPV5. Both mechanisms augment calcium influx from the lumen. FGF23 is involved mainly in phosphate metabolism. Interestingly, recent experimental findings have demonstrated that FGF23 increases the expression of the calcium channel, TRPV5, on the apical membrane of the DT. This suggests that FGF23 may enhance calcium reabsorption in DT, similarly to vitamin D, PTH, and Klotho (25).

Inhibition of NaCl uptake in DCT by thiazides that bind to NaCl cotransporter leads to hypocalciuria. Studies suggest that suppression of NaCl transport into the DCT cells results in hyperpolarization of the cell that activates the TRPV5, thus promoting calcium entry into the cytosol. In parallel, the thiazide-induced fall in intracellular sodium concentration facilitates the action of NCX to enhance the exit of calcium from the

cytosol to the extracellular basolateral compartment. Also, amiloride-, triamterene-, and spironolactone-induced inhibition of sodium reabsorption produces hypocalciuria in a similar fashion. However, more recent studies suggest that the effect of thiazides on tubular calcium reabsorption is primarily mediated by increasing proximal tubule reabsorption due to hypovolemia induced by thiazides (26). *WNK4* that is a negative regulator of NaCl cotransporter in the DT enhances TRPV5-medated calcium transport. Inactivating mutation of WNK4 causes familial hyperkalemic hypertension (PHAII, Gordon syndrome) and leads to reduced calcium reabsorption and hypocalciuria (27).

High extracellular pH stimulates the activity of TRPV5, whereas low pH suppresses its activity. Consequently, pH-dependent inhibition of ECaC1 in acidosis may contribute to renal calcium wasting in this condition (21).

Changes in the filtered load of calcium also may affect the excretion of this ion. Thus, hypocalcemia is associated with a low urinary calcium excretion, regardless of its cause. A micropuncture study in dogs demonstrated that elevation of plasma calcium from a low to a normal level inhibits calcium reabsorption in the loop of Henle independent of PTH. The renal capacity to excrete calcium may be severely compromised by a reduction in glomerular filtration rate (GFR) and ECF volume depletion. The reduced absolute and fractional excretion of calcium in early chronic renal failure when the GFR is only moderately reduced is not well understood (28,29). Two factors might contribute to this observation: secondary hyperparathyroidism and abnormalities of vitamin D metabolism with reduced intestinal absorption of calcium (28). In more advanced renal failure, fractional excretion of calcium is enhanced and correlates with the fractional clearance of sodium, suggesting that the renal handling of both ions may be altered by a similar mechanism at this stage of renal insufficiency (28).

Acute and chronic loads of phosphorus may decrease urinary excretion of calcium. It has been proposed that the reduced urinary calcium excretion is because of an increased deposition of mineral, either in the bone or in other tissues. The hypocalciuria following oral phosphates has been used in the treatment of renal calculi.

Phosphate depletion leads to an increased urinary excretion of calcium, although the mechanism for this effect remains to be defined. The possible role of secondary hypoparathyroidism was not supported by studies in animals in which parathyroidectomy did not alter substantially the hypercalciuric response to phosphate depletion. In rats, the hypercalciuric

response to phosphate depletion is associated with increased intestinal absorption of calcium. A relevant finding is that phosphate depletion may enhance renal conversion of 25-hydroxy-cholecalciferol or 25-hydroxyvitamin D_3 (25[OH]D_3) into 1,25$(OH)_2D_3$, which stimulates intestinal absorption of calcium. Both metabolic and renal tubular acidosis are associated with an increased urinary excretion of calcium.

URINARY EXCRETION OF PHOSPHORUS

About 85% of serum inorganic phosphorus is filtered, and the ratio of $H_2PO_4^-$ to HPO_4^{2-} depends on the pH. The proximal convoluted and straight tubules are the major sites of phosphorus reabsorption. According to micropuncture studies, when glomerular filtrate reaches the late proximal tubule, 70% of filtered phosphorus is reabsorbed. No phosphate is reabsorbed in the loop of Henle. Evidence was obtained that 10% of the filtered load may be absorbed beyond the early DCT. The tubular reabsorption of phosphate is a saturable process and displays a tubular maximum (Tm) reabsorptive capacity.

Phosphorus enters the brush-border membrane of the proximal tubule via the NaPi cotransport against a steep electrochemical gradient. This is energized by the sodium gradient generated by the basolateral sodium pump. Phosphorus moves out of the cell at the basolateral membrane mostly by a sodium-dependent transport (70%) and partly (30%) by a sodium-independent anion exchange system. Only the luminal cotransport is controlled by hormonal and other regulatory factors (e.g., PTH).

Sodium-Phosphate Cotransporters

The structure of a class of NaPi cotransporters in the brush-border membrane of the proximal tubule that facilitates the uptake of both sodium and phosphate in this segment of the nephron has been studied extensively. Many of the changes in the efficiency of phosphate transport are brought about by changes in the amount and activity of the NaPi cotransporters in this segment of the nephron.

Three families of NaPi cotransporters have been identified at the molecular level, named types I, II, and III NaPi cotransporters. Despite functional similarity, there is very little identity among them. Types I and II were cloned from rat and human renal mRNA by expression cloning strategy, whereas type III cotransporters were first identified on the basis of their function as viral cell surface receptors. PiT-1 cell surface receptor

for gibbon ape leukemia virus, *Glvr-1*, and, PiT-2 cell surface receptor for rat amphotropic virus, *RAM-1*. In this regard, they are similar to CD_4 that is a surface receptor for HIV. Types I and II cotransporters are expressed predominantly in the kidney and localized to the brush-border membrane of the proximal tubular cells.

Type 1 cotransporter was the first member to be cloned from a rabbit's kidney. Its physiologic role, however, has not been well delineated. It is both a NaPi cotransporter and a chloride channel. As opposed to type II, type I produces electrogenic transport only at high concentrations of extracellular phosphorus concentrations. Studies suggest that type I functions as channel permeable not only to phosphorus and chloride but also to organic anions.

Type III is ubiquitously expressed and was initially assigned to fulfill a function of housekeeping NaPi cotransporters with no role as transcellular transporters. They have been assigned the role of supplying the basic cellular metabolic needs for phosphorus and as such were assumed to be located basolaterally in the kidney. The deeply ingrained notion that type III plays no role in renal transtubular vectorial transport was recently reassessed. New experimental data advanced evidence that PiT-2, a member of type III, which is localized to the brush-border membrane in proximal tubule epithelia, is a novel mediator of phosphorus reabsorption in the proximal tubule and is regulated by dietary phosphorus (30).

Type II cotransporters are the most abundant of the transporters and are also the major target for regulation by metabolic and hormonal factors including PTH, phosphatonins such as fibroblast growth factor 23 (FGF23), vitamin D, and dietary phosphate. Type II is largely responsible for renal phosphate reabsorption, as indicated by knockout experiments. Targeted inactivation of type II (Npt_2) in mice leads to severe phosphate wasting, 70% to 85% reduction in phosphate reabsorption, hypercalciuria, and skeletal abnormalities. It has been generally assumed that NaPiIIc accounts for the remaining 15% to 30% of phosphorus transport capacity. This view has been questioned by recent experiments that suggest that Npt2c in mice do not play a role in phosphorus transport. Npt_2c knockout mice do not develop phosphaturia or hypophosphatemia. The Npt_2c knockout mice exhibited hypercalcemia, hypercalciuria, elevated plasma $1,25(OH)_2D_3$, and no bone disease (31).

Three isoforms of rat type IIa cotransport have been described (32,33). They appear to be products of alternative splicing, and are designated NaPiIIα, NaPiIIβ, and NaPilly. These isoforms are expressed as proteins at

the brush-border membrane. None of the three spliced isoforms induced expression of NaPi transporter when injected into oocytes. However, when NaPiII*γ* cRNA was coinjected with the type IIa transporter into oocytes, it completely abolished the transport function of the type IIa cotransporter. Thus, alternative splicing may exert a great impact on phosphate transport in the proximal convoluted tubule. The physiologic impact of the aforementioned finding remains to be determined.

Type II cotransporters mediate both electrogenic divalent phosphate transport of $HPO_4^-/3Na^+$ by NaPiIIa, and electroneutral divalent phosphate transport $HPO_4^-/2Na^+$ by NaPiIIc. Type III cotransporters mediate monovalent phosphate electrogenic transport of $H_2PO_4^-/2Na^+$ by PiT-2. NapiIIa exhibits the fastest response to dietary phosphorus.

The function of NaPiIIa is closely linked to Na^+/H^+ exchanger regulating factor-1 (NHERF1), a membrane scaffold protein that plays a crucial role in binding to and anchoring NaPIIIa to the apical membrane. Phosphorylation of NHERF1 by PTH leads to reduced binding of NaPiIIa followed by its endocytosis, internalization, and degradation. This results in decreased phosphorus absorption and increased urinary excretion.

By contrast to rodents, where NaPiIIa has been the principal player in phosphorus absorption, in humans its role is less apparent. There are indications that NaPiIIc may be the dominant phosphorus transporter in humans. Loss-of-function mutation of the NaPiIIc gene is associated with excessive urinary wasting of phosphorus in patients with hereditary hypophosphatemic rickets with hypercalciuria, a human phenotype similar to that of NaPiIIa gene knockout rodents. Mutation of NaPiIIa as a cause of hypophosphatemia in humans has not been reported yet. The above observations underscore the importance of species differences in the regulation of physiologic process.

The essential role of NaPiIIa in tubular phosphorus in vivo has been challenged when exploring the response to intravenous bolus injection of PTH in parathyroidectomized rats. As expected, the phosphaturic effect of PTH was associated with a striking downregulation of NaPiIIa protein expression in brush-border membrane. However, a complete recovery of tubular phosphorus reabsorption that followed was not associated with a membrane recovery of the phosphorus transporter, suggesting that other transport mechanisms may fully compensate for the lack of NaPiIIa (34).

Dietary and Metabolic Factors

Urinary excretion of phosphorus depends on oral phosphorus intake to a great extent. Increased dietary phosphorus is associated with increased total and fractional urinary excretion of phosphorus. This may occur even in the absence of detectable changes in the serum level and filtered load of phosphorus. The state of parathyroid activity seems to play an important role in this phosphaturic response to phosphate load; in fact, this response has been used as a diagnostic test for hyperparathyroidism. Oral intake of 3 g of elemental phosphorus has been reported to increase the excretion of phosphorus to the maximum of 35% of the filtered load in normoparathryoid subjects, but the fractional phosphate excretion exceeds 35% in hyperparathyroid patients. However, although the presence of parathyroid hyperactivity intensifies with phosphaturic response to a phosphate load, the phosphaturic response may be observed in hypoparathyroid patients as well.

Phosphate depletion resulting from phosphate-deficient diets or intestinal phosphate losses is associated with a decrease in urinary excretion of phosphate to negligible amounts. This avid reabsorption of phosphorus is reversed by fasting and acidosis. Animal experiments suggest that increased insulin secretion during phosphorus deprivation contributes to the decreased urinary excretion of phosphorus in the urine.

Animal experiments demonstrated that a low phosphate diet increases the apical expression of type II cotransporters. Metabolic acidosis increases urinary phosphate excretion at the level of brush-border membranes, and NaPiII abundance is reduced. Animal experiments demonstrated that growth hormone, thyroid hormone, insulin, and insulin-like growth factor increase phosphorus reabsorption and upregulate NaPiIIa expression.

Acute expansion of ECF volume with intravenous saline increases the urinary excretion of phosphorus; conversely, acute depletion of ECF volume tends to decrease urinary phosphorus (35). However, the effect of chronically increased oral intake of sodium chloride on urinary phosphorus excretion and phosphorus balance is unknown. In this regard, patients with primary hyperaldosteronism showed no changes in urinary phosphorus excretion but exhibited hypercalciuria.

A high oral intake of calcium is associated with a decreased urinary excretion of phosphorus. Two factors may account for this observation. First, calcium may depress intestinal absorption of phosphorus by forming nonabsorbable complexes with phosphorus. Second, large amounts of oral calcium may suppress the secretion of PTH and reduce urinary excretion of phosphorus. In contrast to its effect when given by the oral route, an

intravenous load of calcium produces an acute increase in serum phosphorus concentration and augments excretion of phosphorus in the urine (2). The rise in serum phosphorus has been attributed to a direct effect of hypercalcemia, namely, promotion of the release of intracellular phosphorus into the circulation (2). This transient phosphaturia is followed by a substantial fall in urinary phosphorus excretion owing to suppression of parathyroid activity (7). In addition, hypercalcemia may exert a direct effect on the kidney, enhancing tubular reabsorption of phosphorus independent of parathyroid activity (36). This effect may be mediated by CaSR stimulation in the proximal tube (37). In contrast to this observation, however, is the finding that restoration of normocalcemia with intravenous calcium in patients with hypoparathyroidism is associated with increased urinary excretion of phosphorus. Likewise, the enhanced excretion of phosphorus that follows the administration of vitamin D to patients with hypoparathyroidism may be at least partly attributable to the restoration of the serum calcium level to normal. A recent study demonstrated that vitamin D downregulates NaPiIIa abundance in parathyroidectomized rats (38).

Acute loads of phosphorus in parathyroidectomized animals produce a net decrease in tubular reabsorption of phosphorus despite a markedly increased filtered load. This change has been linked with the attendant fall in serum calcium concentration and indeed may be reversed by maintaining a constant calcium level (39). This and the foregoing observations show the dependence of renal handling of phosphorus on serum levels of calcium and emphasize the complexity of their interrelationship. A rich phosphate diet in experimental animals downregulates the apical tubular expression of the NaPiII protein.

States of rapid catabolism with increased destruction of body tissues and metabolic acidosis are associated with hyperphosphatemia and phosphaturia. Similarly, cytolysis associated with the administration of cytotoxic agents to patients with neoplasms, especially neoplasms of lymphatic origin, is followed by severe hyperphosphatemia, phosphaturia, and hypocalcemia. Conversely, rapid regrowth of lymphatic tumors may lead to hypophosphatemia of marked degree because of incorporation of phosphorus in the tumor (40).

Intravenous administration of glucose has a dual effect on phosphorus metabolism. First, intravenous glucose tends to lower serum phosphorus, probably by incorporating phosphorus into the intracellular pool during the process of glucose phosphorylation. Second, glucose appears to have a direct renal effect in that it suppresses the reabsorption and increases the

urinary excretion of phosphate. The competition between glucose and phosphate for transport across the epithelium of the proximal tubule has been demonstrated in studies with isolated renal tubules (41). This competition may be most important in states of massive glucosuria with uncontrolled diabetes mellitus.

Most diuretic agents acutely increase urinary phosphorus excretion. However, with the development of ECF volume depletion, the phosphaturic response of diuretics is blunted and may be restored with replacement of urinary losses of sodium and water. Neither the phosphaturic effect of thiazide nor that of acetazolamide seems to be dependent on the presence of parathyroid glands; however, the phosphaturic effect of these diuretics is linked to their ability to inhibit the enzyme carbonic anhydrase. Acidosis increases and alkalosis reduces urinary excretion of phosphorus.

Denervation of kidneys leads to an increase in urinary excretion of phosphorus because of an increased production of dopamine and decreased α- and β-adrenergic renal receptor activity. This denervation-related phosphaturia may contribute to renal losses of phosphorus after kidney transplantation.

Recent experiments in intact and parathyroidectomized rats demonstrated a rapid phosphaturic response to duodenal load of phosphate. Furthermore, protein extracts from homogenates of small intestine that were infused into animals elicited a phosphaturic response. As per suggestions based on the aforementioned observations, the intestine has luminal “sensors of phosphate” that sense increased luminal phosphate concentration and release a substance into the circulation that inhibits renal phosphate reabsorption. The nature of this substance remains to be defined (42).

Regulation of Serum Calcium and Phosphorus Concentration by Hormonal Factors Vitamin D and Its Metabolites

The term “vitamin D” was first introduced by McCollum in 1922 for the antirachitic factor isolated from cod liver oil (43). There are two naturally occurring sterol precursors of vitamin D, namely, ergosterol, which is present in plants, and 7-dehydrocholesterol, which is found in animals and humans. Under exposure to ultraviolet irradiation, ergosterol is converted

into ergocalciferol (calciferol), which is known as vitamin D_2. Vitamin D_1 is not one compound but a mixture of many sterols with antirachitic activity.

The main source of vitamin D in humans is endogenous vitamin D_3, produced by ultraviolet irradiation of 7-dehydrocholesterol in the skin. Areas of skin in most adults contain 3% to 4% of 7-dehydrocholesterol, which is located beneath the stratum corneum. Therefore, excessive amounts of pigment in the skin may interfere with the production of vitamin D_3. The cutaneous synthesis of vitamin D_3 is quite complex. Previtamin D_3 is formed from its precursor 7-dehydrocholesterol. The preceding conversion depends on the levels of 7-dehydrocholesterol and is mediated by initial exposure to ultraviolet light. However, prolonged exposure to ultraviolet light may inactivate previtamin D_3 and transform it to the inert photoproducts, lumisterol and tachysterol. The level of 7-dehydrocholesterol decline with age; therefore, older age predisposes to vitamin D deficiency. Vitamin D_3, also known as cholecalciferol, is formed from previtamin D_3 by thermal isomerization of 2 to 3 days in the skin and also is rapidly degraded by sunlight. Therefore, excessive exposure to sunlight cannot cause vitamin D intoxication because sunlight destroys any excess of vitamin D_3 produced in the skin. Ten to fifteen minutes of exposure to sunlight can provide sufficient amounts of vitamin D_3 for several days' consumption.

The main source of exogenous vitamin D in the United States is milk, which contains about 400 units of vitamin D_2 in each quart. The daily requirement of vitamin D in infants is about 400 units; in older age groups, the requirement is lower, as low as 70 units/day. This modest estimate has been recently challenged because of the high frequency of vitamin D deficiency in the adult and elderly population. Accordingly, higher intake of vitamin D in the range of 600 to 800 units/day has been recommended by some investigators (44).

METABOLISM OF VITAMIN D

Cholecalciferol is metabolized in the liver into 25(OH)D_3, which has a more potent antirachitic activity in vivo than the parent compound, vitamin D undergoes 25-hydroxylation in the liver by 25(OH)ase. It has been generally accepted that 25(OH)ase is not a tightly regulated enzyme, but its activity is reduced by 50% in animals receiving vitamin D. However,

DeLuca et al. demonstrated inhibition of hepatic production of $25(OH)D_3$ by $1,25(OH)_2D_3$ in rats, thus suggesting feedback control (45). A decrease in the level of $25(OH)D_3$ in patients consuming anticonvulsive drugs, such as phenobarbital, phenytoin, primidone, carbamazepine, and rifampin, has been attributed to the induction of cytochrome P-450 enzymes, which leads to increased turnover of vitamin D, including catabolism resulting in vitamin D deficiency and bone disease (46). After enterohepatic circulation, $25(OH)D_3$ is further metabolized in the kidney into $1,25(OH)_2D_3$, which is the most active metabolite of vitamin D. On a weight basis, it is 10 times more effective than vitamin D_3 in curing rickets and 100 times more potent than $25(OH)D_3$ in stimulating calcium mobilization from the bone. When plasma calcium and phosphate levels are normal, 25(OH)D-1α(OH)ase activity in the kidney is reduced, and instead 25(OH)D-24(OH)ase activity prevails and metabolizes $25(OH)D_3$ into $24,25(OH)_2D_3$. Calcitriol is an important negative regulator of itself. It exerts a feedback inhibition of 25(OH)D-1α(OH)ase. Current evidence indicates that this inhibition does not reflect a direct action of $1,25(OH)_2D_3$ on 25(OH)D-1α(OH)ase gene promoter but rather it is an indirect effect. $1,25(OH)_2D_3$ appears to inhibit the known PTH-induced activation of 25(OH)D-1a(OH)ase gene promoter via cyclic-AMP (cAMP). It is the PTH-generated cAMP that induces directly the gene promoter of 25(OH)D-1α(OH)ase resulting in increased production of $1,25(OH)_2D_3$ from 25(OH) D_3 (47). In this regard, previous experiments advanced evidence that vitamin D blocks the formation of cAMP by PTH in the kidney, both in vivo and vitro. The aforementioned findings shed light on the mechanism by which $1,25(OH)_2D_3$ suppresses the activation of 25(OH)-1α(OH)ase (48).

It has been shown that $25(OH)D_3$ in complex with its carrier protein, the vitamin D-binding protein, is filtered through the glomerulus and reabsorbed in the proximal tubules by the endocytic receptor megalin. Endocytosis is required to preserve $1,25(OH)_2D_3$ and deliver it to the cells as the precursor for the generation of $1,25(OH)_2D_3$. These findings contradict the previously held view that $1,25(OH)D_3$ was free, and as such diffused from the circulation across the basolateral surface of proximal tubular cells into the cytosol, to be converted by 25(OH)D-1α(OH)ase to $1,25(OH)_2D_3$. Megalin knockout mice (megalin-1-mice) are unavailable to retrieve the $25(OH)D_3$ from the glomerular filtrate and develop vitamin D–

deficiency state and bone disease. Such a role of the tubular reabsorption process is suggested by observations in patients with renal tubular defects. Similar to megalin knockout mice, patients who suffer from Fanconi syndrome are unable to reabsorb filtered macromolecules and exhibit vitamin D deficiency and bone disease (rickets and osteomalacia). Furthermore, it has been shown in patients with different degrees of renal failure that the GFR was directly correlated with plasma concentrations of $1,25(OH)_2D_3$, suggesting that glomerular filtration is one of the determinants of $1,25(OH)_2D_3$ synthesis by kidney (49).

PTH seems to act as a tropic hormone in stimulating the production of $1,25(OH)_2D_3$ in the kidney. Thus, with intact parathyroid glands, changes in serum calcium indirectly regulate renal production of $1,25(OH)_2D_3$ by altering the secretion of PTH. Specifically, hypocalcemia stimulates and hypercalcemic inhibits the synthesis of $1,25(OH)_2D_3$. In addition, there is evidence that calcium acts directly to alter renal synthesis of calcitriol. Low serum phosphorus stimulates and high serum phosphorus suppresses the renal synthesis of $1,25(OH)_2D_3$ independent of PTH. Several other factors control the formation of $1,25(OH)_2D_3$. The novel group of humoral phosphaturic factors, termed “phosphatonins,” “FGF23,” and others, decrease $1,25(OH)_2D_3$ by inhibiting the activity of 25(OH)d-1α(OH)ase. Furthermore, FGF23 synthesis is enhanced by $1,25(OH)_2D_3$. It has been proposed that FGF23 is a negative feedback regulator of 25(OH)d-1α(OH)ase.

Growth hormone via increased synthesis of insulin-like growth factor I stimulates the activity of 25(OH)d-1α(OH)ase. Chronic metabolic acidosis in humans increases the serum levels of calcitriol (50). This effect could be mediated by acidosis-induced urinary losses of phosphate, leading to cellular phosphate depletion. Once it is formed, $1,25(OH)_2D_3$ is metabolized to several less active metabolites in target tissues (13,51). These transformations are enhanced by the hormone itself and thus may serve to decrease the biologic activity of the hormone once it has carried out its biologic functions. In addition, $1,25(OH)_2D_3$ is excreted in bile as a monoglucuronide, other polar metabolites, and a 23-carbon acid, calcitroic acid (11,13,52). $1,25(OH)_2D_3$ undergoes enterohepatic circulation in humans and various animal species. Proximal tubular cells are the major site of calcitriol formation. In addition, calcitriol may be produced in decidual cells, keratinocytes, bone cells, endothelial cells, peripheral monocytes, parathyroids, colon, prostate, breast and activated

macrophages, where it may also exert a local autocrine or paracrine effect. The main aspects of the metabolism of vitamin D are shown in Figure 6-2.

Dihydrotachysterol (DHT_3) is an analog of vitamin D used in the treatment of hypoparathyroidism. At high doses, it is more effective than vitamin D in mobilizing calcium from the bone, but in low doses it is less effective in curing rickets. DHT_3 undergoes hydroxylation in the liver to $25(OH)DHT_3$, which is the active form of DHT_3. Thus, DHT_3 does not require the presence of the kidneys for the synthesis of its active metabolite. 1α-Hydroxycholecalciferol ($1\alpha[OH]D_3$) is a synthetic sterol that undergoes 25-hydroxylation in the liver to $1,25(OH)_2D_3$ and, like DHT_3, does not require the presence of renal tissue for its conversion into the active form of vitamin D_3. Calcitriol stimulates the metabolic clearance of $25(OH)D_3$. Increased calcitriol formation with increased serum concentration leads to a fall in the serum concentration of $25(OH)D_3$ (53).

EFFECT OF VITAMIN D ON INTESTINAL ABSORPTION

Vitamin D_3 stimulates intestinal absorption of calcium and phosphorus. The effect of vitamin D on calcium absorption becomes measurable several hours after its administration and is blocked by actinomycin D. Circulating calcitriol is a major regulator of intestinal calcium absorption. It exerts its effect mainly by a genomic mechanism mediated by binding to cytosolic vitamin D receptors (VDRs). The calcitriol–VDR structure complexes with retinoic X receptor (RXR) in the target cell nucleus, interacts with specific DNA sequences of calcitriol-responsive genes, and modulates transcriptional and posttranscriptional synthetic pathways. $1,25(OH)_2D_3$ complex with its specific cytosolic VDR promotes mucosal epithelial calcium uptake by induction of the apical calcium channel TRPV6. This is the rate-limiting step in calcium transport. $1,25(OH)_2D_3$ produces a cytosolic calcium-binding protein calbindin D that facilitates transcellular calcium movement, and it upregulates the basolateral PMCA1 that pumps calcium out of the cell.

The promotion of intestinal calcium absorption by vitamin D, at least partly, may be a rapid nongenomic response; the putative membrane receptors for $1,25(OH)_2D_3$ may mediate this process. The active extrusion of calcium at the basolateral side by plasma membrane-bound Ca^{2+} ATPase operates also in the absence of vitamin D. However, $1,25(OH)_2D_3$ has been shown conclusively to stimulate the activity and promote the

synthesis of the plasma membrane calcium pump. The increased synthesis of calcium-binding protein within the intestinal cell and the synthesis of an increased number of calcium pump units enhance the extrusion of calcium from within the intestinal cell into the ECF space.

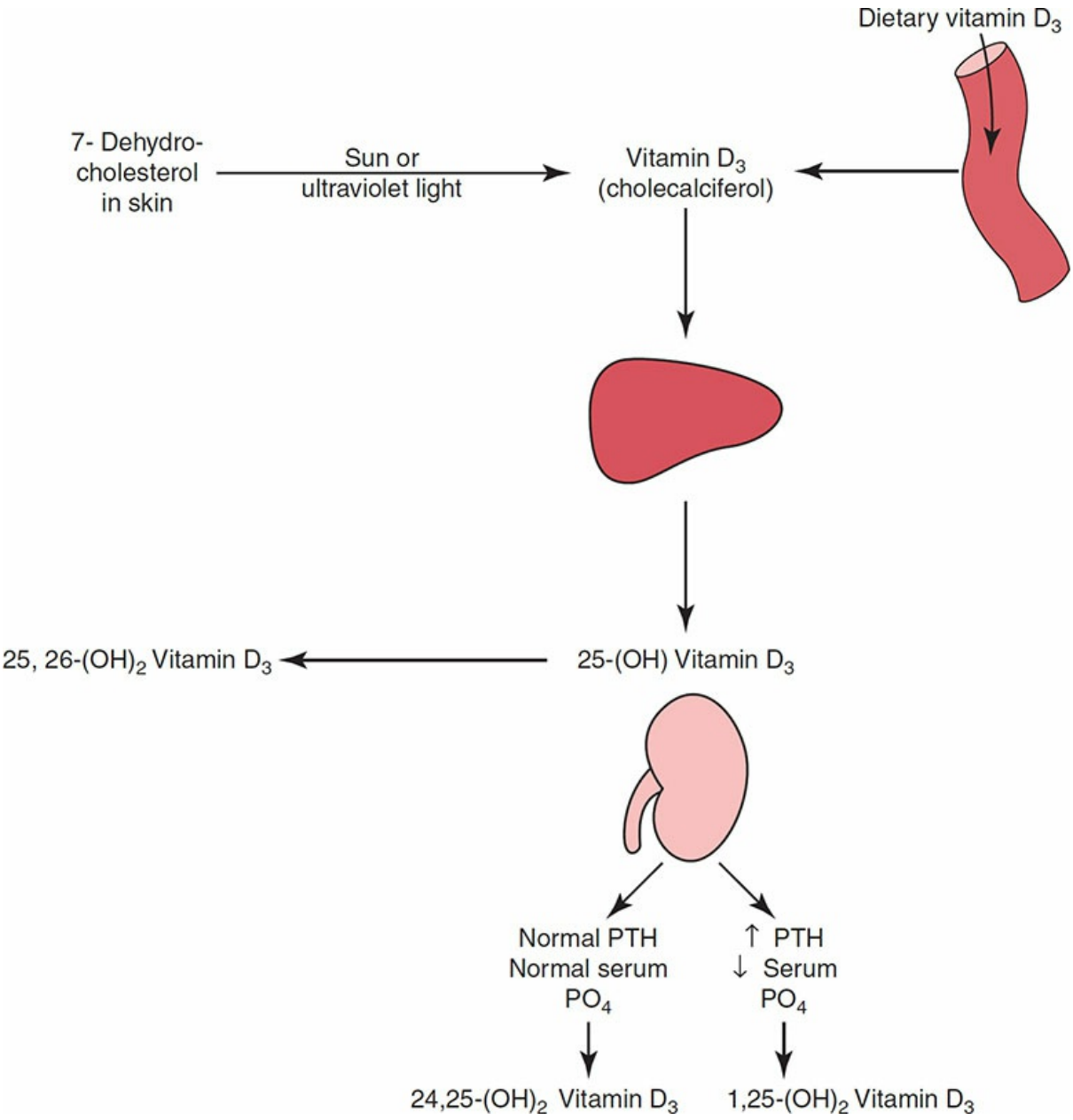


Figure 6–2 Metabolism of vitamin D. The major source of vitamin D_3 is its production in the skin; the other important source is diet. PTH, parathyroid hormone.

Vitamin D Receptors

In addition to intestinal mucosa, calcitriol receptors are present on osteoblasts, monocytes, human breast cancer cells, parathyroid gland, epidermal cells, and cerebellum. Their role will be discussed in other

sections (13,51,52).

EFFECT OF VITAMIN D ON BONE METABOLISM

Vitamin D promotes mineralization of the organic bone matrix. This action appears to be at least partly secondary to the effect of vitamin D on enhancing the intestinal absorption of calcium and phosphorus and thus maintaining their normal ECF concentrations. Some evidence supports a direct role of vitamin D in bone accretion (54). However, it has shown that vitamin D-deficient osteomalacia may be cured with the intravenous administration of calcium and phosphorus despite the persistence of a vitamin D–deficiency state (55).

Evidence suggests that vitamin D and its metabolites $25(OH)D_3$ and $1,25(OH)_2D_3$ mobilize calcium and phosphorus from bone, an effect that has been demonstrated both in vivo and vitro (56,57). Therefore, this action of vitamin D may increase serum calcium concentration independently of its enhancement of the intestinal transport of calcium. Studies in animals have shown that vitamin D stimulates both osteocytic and osteoclastic bone resorption, and this action does not require the presence of PTH (56). Calcitriol induces differentiation of monocytic cells into mature osteoclasts, and it increases the number of osteoclasts. $1,25(OH)_2D_3$ interacts with VDRs in osteoblasts to induce the expression of the receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL). RANKL binds to RANK on the plasma membrane of preosteoclasts and transforms them into mature osteoclasts. Osteoclasts dissolve the bone and release calcium and phosphorus into the circulation. Calcitriol increases osteoblast size and increases the synthesis of alkaline phosphatase and the blood level of osteocalcin. Interestingly, in vitro studies suggest that $1,25(OH)D_3$, but not other metabolites of vitamin D, may inhibit bone collagen synthesis (57).

The exact role of $24,25(OH)D_3$ in mineral metabolism is unknown and controversial. Many investigators view it as an inactive waste product of vitamin D catabolism. In animals, the hormone is metabolized to $1,24,25(OH)D_3$ and becomes active in intestinal absorption of calcium. In normal, hypoparathyroid, and anephric humans, however, $24,25(OH)D_3$ acts directly to increase intestinal absorption of calcium, even when given in relatively low doses (58). This effect of $24,25(OH)D_3$ is associated with positive calcium balance without changes in serum concentration or urinary excretion. In view of this observation and the previously reported

effect of $24,25(OH)_2D_3$ to promote the synthesis of protein by chondrocytes, it has been proposed that $24,25,(OH)D_3$ may be the metabolite that is directly involved in a skeletal metabolism (58). Furthermore, it has been reported that $1\alpha(OH)D_3$ alone does not prevent rickets in chicks, whereas $24,25(OH)_2D_3$ alone is effective (59). Experimental studies also have demonstrated that $24,25(OH)_2D_3$ may play an important role in the suppression of bone resorption in rats after nephrectomy (60). In vitro studies demonstrated that $24,25(OH)D_3$ antagonizes the osseous calcium-mobilizing effect of calcitriol (61).

Recent experiments from our laboratory showed that $1,25(OH)_2D_3$ induced a dose-dependent increase in calcium efflux from cultured bone. This increase was completely obliterated by inhibition of protein kinase C (PKC) with either staurosporine or cephalostin c. In cultured rat calvariae, $1,25(OH)_2D_3$ also induced a dose-dependent translocation of PKC from cytosol to membrane. This activation of PKC by $1,25(OH)_2D_3$ occurred following 30 seconds of incubation, peaked at 1 minute, and disappeared by 5 minutes. $1,25(OH)_2D_3$ did not increase cAMP production in similarly cultured calvaria. These results suggest that the action of $1,25(OH)_2D_3$ on calcium flux from bone tissue is mediated by the activation of PKC (62).

Similarly, we have shown in the same experimental model that $24,25(OH)D_2D_3$ induced a dose-dependent flux of calcium into the bone. This effect was mediated by an inactivation of PKC. Thus, the action of $1,25(OH)_2D_3$, which mobilizes calcium from bone, and $24,25(OH)_2D_3$, which inhibits bone resorption, are mediated by activation and inhibition of PKC, respectively (63). It is interesting that other investigators demonstrated that $1,25(OH)_2D_3$ activates PKC, rapidly increases intracellular calcium, and stimulates polyphosphoinositide hydrolysis in colonic epithelia (64). Thus, there is mounting evidence demonstrating a role of PKC activation in mediating the nongenomic effects of vitamin D.

EFFECT OF VITAMIN D ON RENAL HANDLING OF PHOSPHORUS AND CALCIUM

The effect of vitamin D on renal handling of phosphorus has been the subject of numerous investigations. The main difficulty encountered in interpreting the changes in urinary excretion of phosphorus has been related to the calcemic actions of vitamin D, which, by suppressing PTH secretion, indirectly alter renal handling of phosphorus. Consequently, the

enhanced tubular reabsorption of phosphorus following the administration of vitamin D to patients with osteomalacia and rachitic animals with intact parathyroid glands could be accounted for either by inhibition of PTH secretion or a direct tubular action of vitamin D. The results of studies in animals suggest that both $25(OH)D_3$ and $1,25(OH)_2D_3$ acutely enhance tubular reabsorption of phosphorus (65); in rats, this effect requires the presence of either endogenous or exogenous PTH. The antiphosphaturic effect on minimal doses of $1,25(OH)_2D_3$ was demonstrated in chronic studies in vitamin D-deficient rats; this effect was reported to be associated with upregulated NaPiII expression. The effects of $1,25(OH)_2D_3$ are summarized in Figure 6-3.

Recent studies from our laboratory addressed the mechanism(s) underlying the effects of vitamin D on renal handling of phosphate both in acute experiments, using opossum kidney (OK) cell line, and in chronic experiments using metabolic clearances in parathyroidectomized rats infused with $1,25(OH)_2D_3$ without and with PTH via osmotic minipumps over 7 days. The acute studies in OK cells reproduced our previous results in rats (65,66). These experiments demonstrated that treatment for 24 hours with $1,25(OH)_2D_3$ antagonized the effect of PTH to inhibit phosphate uptake in OK cells. This action of $1,25(OH)_2D_3$ was associated with suppressed PTH-induced activation of the second messenger transduction pathway, the adenylate cyclase/cAMP/PKA system. Interestingly, this response to vitamin D was accompanied by a substantial diminution in the expression of the PTH/PTH-related peptide (PTHrP) receptor. The latter could at least partly account for the inhibition of adenylate cyclase-cAMP activation by PTH. Likewise, it is interesting that the observed PTH-triggered rise in intracellular calcium, which presumably resulted from PTH-induced activation of the second signal transduction pathway, namely the activation of PKC, was not altered by $1,25(OH)_2D_3$. In contrast to the acute studies, in chronic experiments, $1,25(OH)_2D_3$ enhanced the phosphaturic effect of PTH despite retaining the concomitant reduction in urinary cAMP excretion. We observed that both NapIII mRNA and NaPiII protein were significantly reduced when $1,25(OH)_2D_3$ was infused alone, when PTH was given alone, and, most strikingly, when both were given together. Furthermore, the observed vitamin D-induced downregulation of the PTH/PTHrP receptor was reversed in the chronic study when PTH and $1,25(OH)_2D_3$ were given together. The latter could contribute at least partly to the enhancement of

PTH-induced phosphaturia by $1,25(OH)_2D_3$. Thus, as opposed to acute conditions where vitamin D blunts the phosphaturic effect of PTH, chronic administration of vitamin D exerts the opposite effect (67). Therefore, it is apparent that the effect of vitamin D on renal handling of phosphate is very complex and depends on many variables.

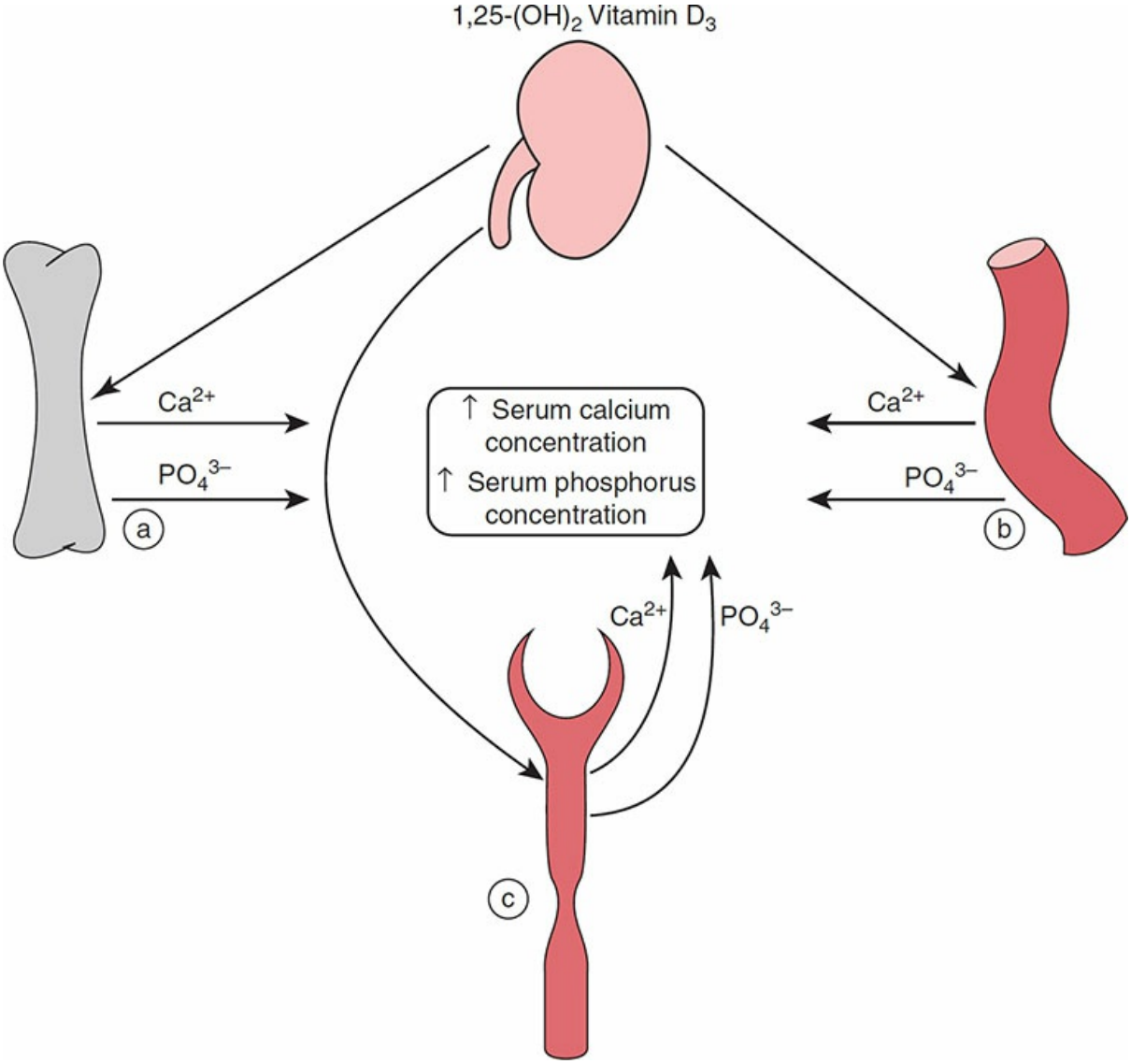


Figure 6–3 Hypercalcemic and hyperphosphatemic effect of $1,25(OH)_2D_3$. Its actions are: (*a*) mobilization of mineral from bone; (*b*) enhanced intestinal absorption of calcium and phosphorus; and (*c*) augmented tubular absorption of phosphorus and calcium. The net physiologic effect is the maintenance of a normal serum calcium and phosphorus product, which allows mineralization of bone.

Large doses of vitamin D cause hypercalciuria, possibly by increasing absorption of calcium from the intestine. In contrast, acute clearance studies in dogs showed an increased renal tubular absorption of calcium after intravenous administration of vitamin D (68,69). Vitamin D,

however, does not appear to be essential for the renal conservation of calcium, because urinary calcium excretion may be reduced to extremely low levels in osteomalacia resulting from vitamin D deficiency (55).

In addition to the effect of vitamin D on the intestine, bone, and kidney, vitamin D acts directly on parathyroid tissue to suppress secretion of PTH. Studies in rats in our laboratory demonstrated that physiologic amounts of $1,25(OH)D_3$ inhibit the levels of PTH mRNA, independent of serum calcium levels (70). This action of calcitriol is mediated by the VDR. Calcitriol acts on at least two negative regulatory elements upstream in the 5′ flanking region of the PTH gene to suppress transcription. Furthermore, calcitriol modulates secretion and synthesis of PTH by increasing gene expression of the VDR in the parathyroid gland (53).

In view of this information, a feedback loop may be formulated that has the following sequence: PTH stimulates the formation of $1,25(OH)_2D_3$, and $1,25(OH)D_3$ closes the negative feedback loop by suppressing the secretion of PTH. Thus, among other functions, $1,25(OH)_2D_3$ may have a modifying effect on the secretion of PTH. FGF23 inhibits renal synthesis of $1,25(OH)_2D$, and indirectly, via lowering the active vitamin D metabolite, increases PTH secretion. This effect has been observed in chronic kidney disease (CKD). This assumption is supported by the observation that the FGF23 rise in CKD precedes that of PTH and the decrease in $1,25(OH)_2D_3$ (53).

Vitamin D activity in the serum and in other tissues may be measured by both bioassay and radioimmunoassay techniques. The radioreceptor assay can determine the serum levels of various metabolites. These competitive protein-binding assays have great potential importance in determining the mechanisms underlying clinical disorders secondary to abnormalities in vitamin D metabolism.

Parathyroid Hormone

PTH is a single-chain polypeptide of 84 amino acid residues (mol wt 9,500) with biologic activity in the *N*-terminal 1 to 34 region of the molecule. The biosynthesis of the hormone starts with prepro-PTH, a 110 amino acid chain polypeptide that is the translation product of PTH mRNA. Pro-PTH is produced after cleavage of 21 amino acids. PTH is produced after additional cleavage and stored in secretary droplets. The amount of stored hormones is sufficient for basal secretion over 5 to 6

hours and 2 hours of augmented secretion. Thus, the synthesis is closely linked to secretory activity.

PTH plays a central role in the physiologic regulation of serum calcium concentration. Serum calcium concentration is maintained within a very narrow range, primarily because of a feedback mechanism in which minimal changes in ionized calcium alter the secretory rate of PTH, which then restores the ionized calcium to its normal concentration by its action on bone. Serum concentration of phosphorus is not feedback regulated; therefore, it varies over a relatively wide range. Recent studies, however, suggest that changes in serum phosphate may be involved in the regulation of PTH secretion. There is a direct relationship between serum phosphate level and the secretion of PTH. Thus, high serum phosphate levels increase and low levels decrease the synthesis and secretion of PTH. This appears to be a posttranscriptional effect. This effect appears to be independent of changes in serum calcium and vitamin D (71,72).

It is apparent that serum-ionized calcium is the single most important physiologic factor controlling the secretory rate of PTH. A sensitive inverse relationship has been demonstrated between ionized calcium and serum level of PTH (8). The parathyroid cells have a cell surface sensing mechanism to extracellular calcium concentration that also recognizes other divalent and polyvalent cations, such as magnesium and neomycin. This mechanism is based on a CaSR. The CaSR is a membrane of the G protein-coupled receptor family that responds to increased extracellular calcium by triggering the phospholipase C pathway and elevating inositol triphosphate, diacylglycerol, and intracellular calcium concentration. The increased intracellular calcium inhibits PTH secretion from parathyroid cells. The CaSR expressed in the parathyroid, thyroid, and kidney was cloned and characterized (23). Recently, another possible CaSR that is distinct from the G protein-coupled receptor was identified. This receptor (CAS) is a large protein known as gp330 megalin and is a member of the low-density lipoprotein receptor superfamily. It was identified in parathyroid tissue, but its role in parathyroid physiology is unknown. In addition to its acute effect on PTH secretion, chronic changes in serum-ionized calcium concentration, both hypercalcemia and hypocalcemia reduce or increase the steady-state level of PTH mRNA and synthesis of PTH. In vivo, calcitriol causes a 90% decrease in prepro-PTH mRNA at 48 hours; the effect starts after 2 hours. As opposed to calcium, which exerts both an acute and a chronic effect on PTH secretion, calcitriol does not have an acute effect on PTH secretion, but there is a decrease in PTH secretion after 12 to 24 hours (53).

An increase in PTH secretion also has been observed in cows during the administration of epinephrine, raising the possibility that the autonomic nervous system may play a role in controlling PTH secretion. An aberration in extracellular pH affects PTH secretion rate. A decrease in pH inhibits CaSR activity, leading to an increase in PTH secretion, whereas alkalinization enhances CaSR activity leading to a decrease in PTH secretion. In animal experiments, metabolic acidosis predisposes to elevated PTH levels with concomitant hypercalcemia, whereas alkalosis lowers PTH levels (73).

Peripheral Actions of Parathyroid Hormone and Parathyroid Hormone-Related Peptide

The peripheral actions of PTH and PTHrP on bone and kidney involve binding to cell surface receptors followed by activation of two pathways of signal transduction. Thus, PTH stimulates both the adenylate cAMP-PKA pathway and phospholipase C, which in turn leads to activation of PKC by diacylglycerol and an increase in intracellular calcium by inositol triphosphate. The stimulation of these two signaling pathways is mediated by coupling of the hormone-occupied receptor with two distinct G proteins, which link the receptor to effector pathways (74,75). The gene for the human PTH receptor for bone and kidney has been cloned, sequenced, and expressed in African green monkey kidney (cos) cells. The evaluation of the structure and function relationship of the receptor and PTH is interesting. It has been demonstrated that the *N*-terminal fragment PTH sequence 1 to 34 reproduces all physiologic effects of PTH sequence 1 to 84. It has been shown that the amino acid sequences 10 to 15 and 24 to 34 of PTH are necessary for binding to the receptor. With regard to the biologic effects of PTH, it has been shown that the first two *N*-terminal amino acids 1 and 2 are required for the activation of adenylate cyclase–PKA pathway, whereas the amino acids sequence 28 to 34 are required for the activation of phospholipase C–PKC pathway. Indeed, the fragment PTH sequence 3 to 34 was shown in vitro to suppress phosphate transport without activation of adenylate cyclase–PKA pathway (74–76).

Another PTH receptor (type 2) that binds only PTH and does not bind PTH-related protein (PTHrP) has been found in the brain and intestines. The functions of this receptor are unknown.

EFFECT OF PARATHYROID HORMONE ON BONE

PTH plays a major role in bone remodeling. PTH increase bone turnover owing both to increase in osteoclast numbers and resorption and stimulation of bone formation by osteoblast activation.

Its receptors are expressed in bone-forming cells, osteoblasts, and proosteoblasts, but not in osteoclasts. Thus, although PTH acts to increase osteoclastic resorption, it appears that this effect is not mediated via receptors on osteoclasts but are indirect, occurring through the interaction of PTH with receptors on osteoblasts. PTH-activated osteoblasts may enhance recruitment and stimulation of osteoclasts.

PTH augments release of mineral from bone by stimulating both osteocytic and osteoclastic bone resorption and possibly by enhancing calcium transport from the skeletal ECF into the systemic ECF. There is experimental evidence that the latter is a direct effect. The resulting increase in serum calcium concentration may be preceded by a short period of decreasing concentration because of an initial enhanced entry of calcium in bone cells.

The calcemic effect of PTH on bone requires the presence of vitamin D (77,78). The impaired response to PTH in vitamin D deficiency may be because of either some permissive action of the vitamin or the mechanical blocking effect of the osteoid that coats the surface of the mineralized bone and thus prevents access of the PTH. Correction hypocalcemia per se in rats has been shown to restore the responsiveness of the bone to the action of the PTH in states of vitamin D deficiency. This observation is consistent with the possibility that calcium is a cofactor in the skeletal action of PTH. Recently, proposals hold that PTH acts on two distinct cellular systems in the bone: (a) the remodeling system and (b) the calcium mobilization or calcemic-homeostatic system. The remodeling system consists of osteoclasts that resorb old bone and osteoblasts that form new bone. In this system, bone resorption is balanced by bone formation; therefore, no mineral escapes into the circulation. The homeostatic system is based on the action of surface osteocytes and osteocytes occupying lacunar spaces that regulate the movement of calcium between the bone fluid and ECF. This mineral-releasing system is important in everyday regulation of serum calcium and requires $1,25(OH)_2D_3$ in addition to PTH. Recent in vitro studies suggest that the calcium-mobilizing effect of PTH is mediated by activation of the phospholipase C–PKC signal transduction system (79).

EFFECT OF PARATHYROID HORMONE ON THE KIDNEY

The primary renal effect of PTH is to produce phosphaturia by depressing net phosphate reabsorption in the proximal tubule. This tubular effect involves PTH receptor–mediated intracellular formation of messenger cAMP, inositol triphosphate, diacylglycerol, and free cytosolic calcium and activation of PKA and PKC. These inhibit brush-border transport systems including NaPi cotransport and sodium-proton antiport exchange (74). The results of certain studies suggest additional effects of PTH in more distal parts of the nephron on phosphorus absorption (80). Phosphate depletion produces resistance to the phosphaturic action of PTH in rats; however, this has not been shown yet in humans.

Type II Na/Pi cotransporters are believed to represent the major pathway of renal phosphate reabsorption and are the major target with respect to inhibition of proximal tubular reabsorption by PTH. Both acute and chronic administration of PTH downregulate the NaPiII protein in brush-border membrane of proximal tubular cells; however, only chronic administration downregulates NaPiII mRNA. In vivo and in vitro experiments showed that PTH causes retrieval of NaPiII cotransporters from the membrane. After internalization, they are routed to the lysosomes, where they are degraded.

It had been earlier assumed but never proven that NaPiII downregulated by PTH involves phosphorylation of the transporter by cAMP. Recent evidence suggests that PTH induces phosphorylation of the PDZI domain in NHERF1, a scaffold protein that binds to and anchors NaPiIIa to the apical membrane. Phosphorylation of the scaffold protein reduces its bindings to NaPiIIa and leads to NaPiIIa internalization and degradation in the lysosome (81).

Although the effect of PTH in the proximal tubule is to depress calcium reabsorption, its net effect is to decrease urinary calcium excretion in dogs, rats (82), and humans. The net increase in tubular reabsorption of calcium appears to be caused primarily by a distal action of the hormone, where approximately 10% to 20% of filtered calcium is reabsorbed (18). Thus, it appears that both the renal and the skeletal actions of PTH act jointly to increase serum concentrations of calcium. Because PTH may increase bicarbonate, sodium, and amino acid excretion, the hormone does not appear to increase the reabsorption of these substances in the distal nephron. Parathyroidectomy in rats is associated with downregulation of calcium channel TRPV5, calbindin D_{28K}, and expression of basolateral calcium transporters. The exact molecular mechanism involved in PTH-induced increase in calcium transport in DCT and CNT has not been elucidated in detail yet. The classic concept that cAMP stimulates calcium

reabsorption in this nephron segment has been questioned. Recent studies suggest that PTH stimulates calcium reabsorption in the distal nephron, independent of cAMP, via activation of the phospholipase C–PKC signal transduction pathway. Activation of PKC increases cell surface abundance of TRPV5 by inhibiting endocytosis. This mechanism of regulation of PKC may contribute to acute stimulation of TRPV5 and calcium absorption of PTH (83).

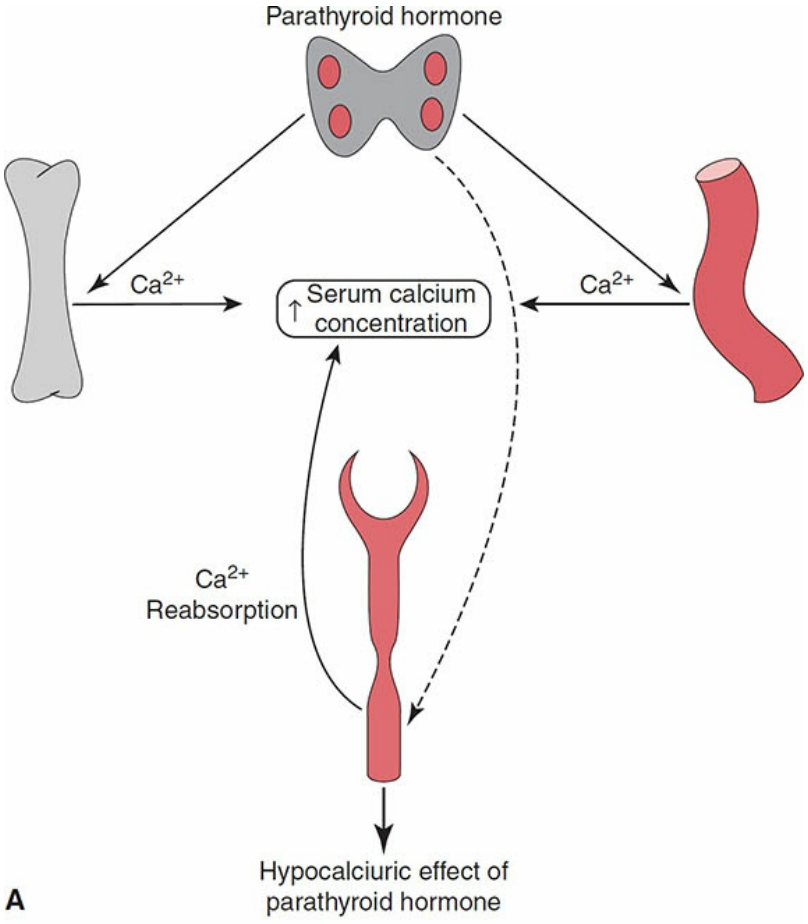
EFFECT OF PARATHYROID HORMONE ON INTESTINAL ABSORPTION OF CALCIUM

A role of PTH in the intestinal absorption of calcium has been suggested by several studies in both animals and humans. However, at present there is no evidence to support a direct action of PTH on calcium transport in the intestine. The fact that PTH enhances the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, which directly acts on the intestinal transport of calcium, may explain the apparent effects of the hormone. Even so, it is obvious that in states of vitamin D deficiency, elevated levels of circulating PTH fail to maintain normal absorption of calcium. Conversely, vitamin D may affect intestinal absorption in the absence of PTH in patients with hypoparathyroidism. The multiple actions of PTH are summarized in Figure 6-4.

RADIOIMMUNOASSAY OF PARATHYROID HORMONE

Radioimmunoassay for circulating PTH was introduced by Berson and Yalow (84) in 1963. Further studies led to the recognition of the heterogeneity of circulating PTH, which apparently represents various molecular species of the hormone. The glandular hormone (mol wt 9,500) consists of 84 amino acids (1–84 sequence) and has two terminals, amino ($-NH_2$) and carboxy (-COOH). The circulating PTH consists of the glandular hormone and its fragments. At least two different molecular species of circulating PTH have been detected by different antisera, one with a molecular weight of 4,500 to 5,000 and one with a molecular weight of 7,000 to 7,500. Structurally, there are two major split products that can be characterized by their terminals. The first product has the *N*-terminal, is the biologically active fragment, and has an amino acid sequence 1 to 34. The second product has the *C*-terminal, is the biologically inactive fragment, and has an amino acid sequence 53 to 84. The level of total circulating immunoreactive PTH that reflects all

molecular species appears to represent the chronic state of parathyroid function and is most useful in the diagnosis of parathyroid abnormalities. The level of the glandular (mol wt 9,500) species represents acute changes in parathyroid activity, such as those that occur after calcium infusion. Although it has been suggested that the level of the *C*-terminal fragment provides the best differentiation between normal persons and patients with hyperparathyroidism, this seems paradoxical in view of the fact that the *C*-terminal is biologically inactive. It should be emphasized that radioimmunoassays that measure the *C*-terminal fragments do not provide a reliable estimate of parathyroid function in patients with renal insufficiency. This is because the clearance of *C*-terminal fragments is delayed in renal failure. Thus, the renal failure in radioimmunoassay of *N*-terminal species of PTH or the intact hormone molecule provides a better indication of parathyroid function. The intact PTH assay employs two antibodies, one binding the *N*-terminal and the second binding the *C*-terminal. One antibody is fixed on beads that are then exposed to serum. After incubation with the tested serum, the beads are separated and exposed to radiolabeled antibodies binding the opposite terminal.


Parathyroid hormone
Ca2+
Serum calcium concentration
Ca2+
Ca2+ Reabsorption
Hypocalciuric effect of parathyroid hormone
A

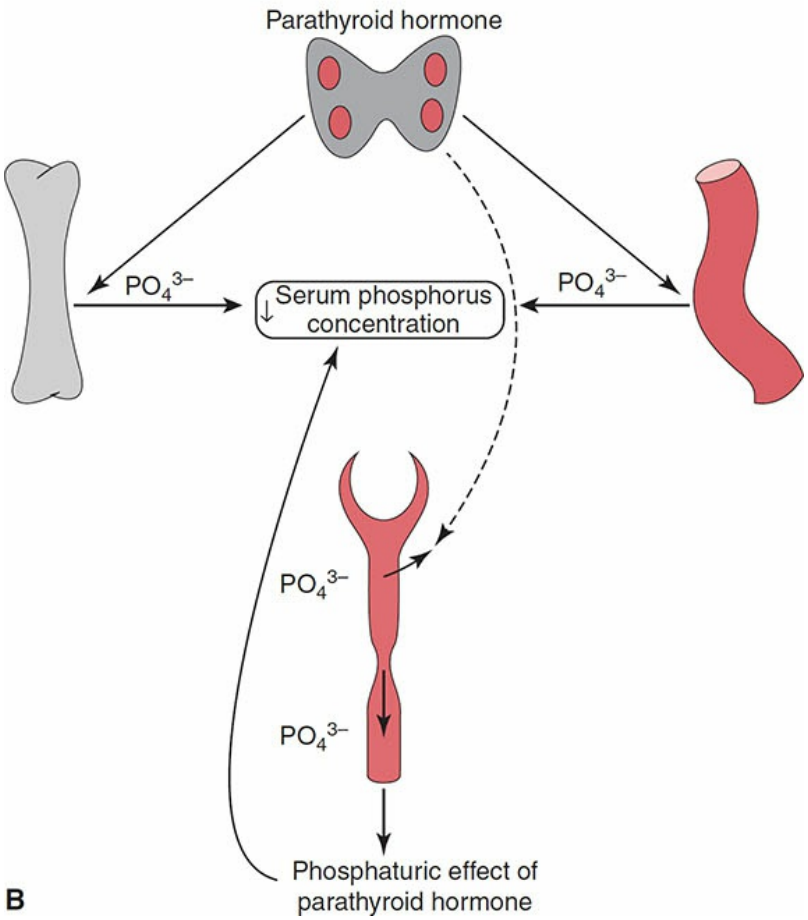

Parathyroid hormone
PO4 3–
Serum phosphorus concentration
PO4 3–
PO4 3–
PO4 3–
Phosphaturic effect of parathyroid hormone
B

Figure 6–4 (**A**) The hypercalcemic effect of parathyroid hormone (PTH) is a summation of mineral mobilization from bone, calcium absorption from the bowel, and distal tubular reabsorption of calcium in the kidney. The effect on the bowel probably is related to PTH-induced renal production of $1,25(OH)_2D_3$. (**B**) The hypophosphatemic effect of PTH is based on its phosphaturic action, which supersedes its effect of mobilizing phosphorus from bone and enhancing phosphate absorption from the intestine. (*Solid lines* represent an enhancement of action; *broken line* represents an inhibition of action.)

Recently, it has been realized that current two-site assays designed to detect both an *N*-terminal and *C*-terminal epitopes of PTH may not be reliable. PTH molecules that are reactive in these assays are considered intact, but some have no bioactivity. For example, a loss of only six amino acids to yield PTH (83–85) eliminates all bioactivity but does not affect the immunoreactivity measured in most of these assays. In fact, about 50% of PTH detected in these assays in the serum of patients with chronic renal failure is not only biologically inactive but also exhibits an antagonistic effect on the biologic activity of 1 to 84 PTH. The newer immunodetection of PTH by whole PTH two-site assay that recognizes the first six amino acids appears to be more reliable in measuring only biologically active PTH.

Calcitonin

The discovery of calcitonin established the presence of a new regulatory system for calcium homeostasis. Calcitonin is a polypeptide with 32 amino acid residues and was isolated from the parafollicular cells of the thyroid gland or ultimobranchial body in a wide variety of species. Hypercalcemia stimulates release of calcitonin, which tends to lower serum calcium concentration (85).

EFFECT OF CALCITONIN ON BONE

The major mechanism by which calcitonin lowers serum calcium and phosphorus concentrations is inhibition of bone resorption. This is associated with decreased osteoclastic activity and decreased urinary hydroxyproline excretion. In organ culture, after prolonged treatment with calcitonin, PTH may overcome the inhibitory effect of calcitonin and induce bone resorption. This phenomenon is termed “escape” and is also

observed in vivo in animals with intact parathyroid glands chronically treated with calcium. An antagonism exists between calcitonin and glucocorticoid hormones, because glucocorticoids interfere with the hypocalcemic action of calcitonin.

The receptors of calcitonin have been cloned from human giant cell tumors of bone, human ovary, and breast cell line (75). The calcitonin receptor is expressed by osteoclasts, as opposed to PTH and calcitriol receptors, which are expressed only by osteoblasts. Similar to PTH receptors, calcitonin receptors couple to two signal transduction pathways, adenylate cyclase–PKA and phospholipase C–PKC via linking with G proteins. Calcitonin acts directly to inhibit osteoclast action on the bone and inhibits osteoclast motility in isolated osteoclast preparations.

EFFECT OF CALCITONIN ON THE KIDNEY

Calcitonin increases urinary excretion of phosphorus, sodium, potassium, and calcium. This effect is independent of PTH. In fact, calcitonin acts to reverse the effect of PTH in two organs. It inhibits bone resorption and increases urinary calcium excretion; both actions tend to lower serum calcium. The phosphaturic action of calcitonin has been found to be associated with an increased urinary excretion of cAMP.

Under normocalcemic conditions, calcitonin, and not PTH, has an important role in the maintenance of serum $1,25(OH)_2D_3$ levels. Calcitonin enhances induction of 25(OH)D-1α(OH)ase transcription and protein expression. Because plasma calcitonin is increased during pregnancy and lactation, it has been proposed that this is the mechanism of increased $1,25(OH)_2D_3$ observed during pregnancy and lactation when calcium requirements are increased (86). Likewise, it is important to remember that experiments conducted in thyroparathyroidectomized animals that are not replaced with calcitonin supplements may invalidate the results.

EFFECT OF CALCITONIN ON INTESTINAL ABSORPTION

The effect of calcitonin on intestinal absorption has not been studied extensively. Preliminary reports indicate, however, that calcitonin has no effect on intestine calcium absorption but may actively decrease the absorption of phosphorus, sodium, potassium, and chloride. The multiple actions of calcitonin are summarized in Figure 6-5.

The development of a sensitive radioimmunoassay for calcitonin has provided the means to study the control of secretion of this hormone. From

a clinical standpoint, the radioimmunoassay serves as a valuable aid in the diagnosis of medullary carcinoma of thyroid, which is a calcitonin-secreting tumor.

Disorders of Calcium and Phosphorus Metabolism Associated with Hypocalcemia

VITAMIN D DEFICIENCY

Hypocalcemia is a common feature in vitamin D deficiency; however, this disorder may be present with a normal serum calcium concentration. For example, vitamin D–deficiency rickets in children evolves over three stages. During the first stage, serum calcium concentration is low, serum phosphorus is normal, and immunoreactive PTH in the serum is normal (76,87). There is no satisfactory explanation for the normal PTH in the presence of hypocalcemia. During the second stage, there is a rise in PTH activity, and serum calcium concentration rises to a normal level as serum phosphorus concentration decreases. In the third stage, which is the most severe, both serum calcium and phosphorus concentrations are low (78). It is unknown whether vitamin D–deficiency osteomalacia in adults shows a similar evolution.

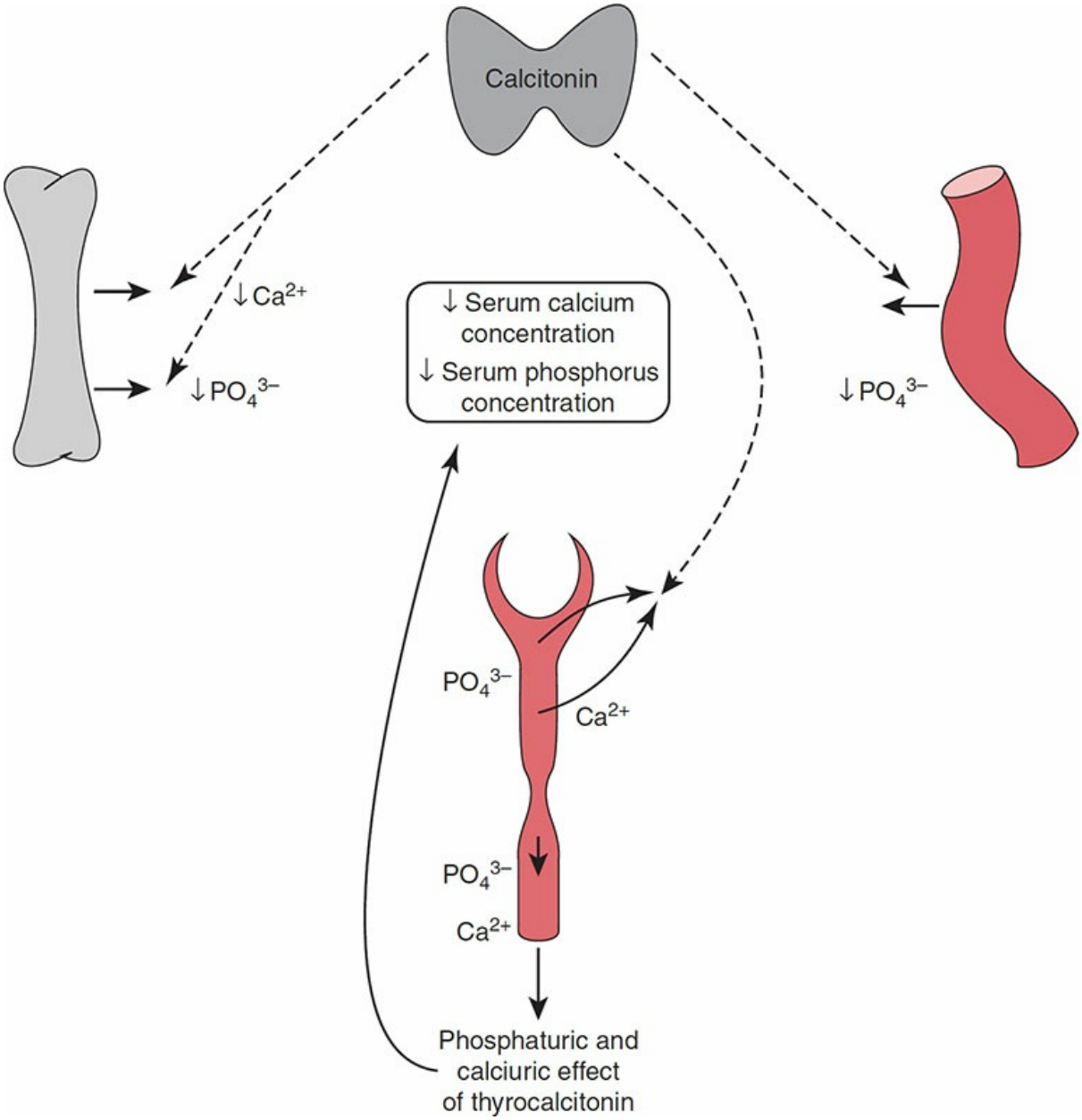


Figure 6–5 The hypocalcemic and hypophosphatemic actions of calcitonin are based on inhibition of mineral mobilization from the bone, decreased tubular reabsorption and increased urinary excretion of calcium and phosphorus, and decreased intestinal absorption of phosphorus. (*Solid lines* represent an enhancing effect of the hormone; *broken lines* represent an inhibitory action.)

Table 6–2 Common Causes of Vitamin D Deficiency

Nutritional	Abnormal metabolism of vitamin D
Malabsorption	Vitamin D-dependent rickets
Following gastrectomy	Ingestion of barbiturates and anticonvulsants
Tropical and nontropical sprue	Renal insufficiency

Chronic pancreatitis	Hepatic dysfunction
Biliary cirrhosis	Calcium deprivation
Ingestion of cathartics	Renal losses of vitamin D
Intestinal bypass	Nephrotic syndrome
Anticonvulsant therapy	Fanconi syndrome

The various common etiologies of vitamin D deficiency are listed in Table 6-2. Because vitamin D is fat-soluble, nutritional osteomalacia usually is associated with a deficient intake of food products containing fatty substances (78,88). Partial gastrectomy may lead either to a simple dietary deficiency of vitamin D as a result of avoiding fatty foods or malabsorption of vitamin D. Small bowel disease may produce both malabsorption of vitamin D and mucosal resistance to its action. Bile salt deficiency interferes with vitamin D absorption, and hepatocellular failure may interfere with its metabolism. Factitious diarrhea caused by prolonged ingestion of laxatives also may cause vitamin D deficiency. Likewise, nephrotic syndrome is associated with urinary losses and low levels of circulating $25(OH)D_3$ (89).

In addition to vitamin D deficiency resulting from nutritional and GI causes, a group of disorders has been identified in which the deficiency is caused by an abnormal metabolism of vitamin D. Vitamin D-dependent rickets is an inherited autosomal recessive disorder. It appears during early infancy and responds to pharmacologic doses of vitamin D and physiologic doses of calcitrol. This disorder represents an inherited deficiency in the kidney enzyme $25(OH)_2D_3$-1α(OH)ase, which converts $25(OH)_2D_3$ into $1,25(OH)_2D_3$ (88).

25(OH)D-1α(OH) is a mitochondrial cytochrome P-450 enzyme that functions in proximal tubular cells. Although it is a key enzyme in vitamin D metabolism, its cloning was difficult because of the low level of gene expression. The recent cloning of the human 25(OH)D-1α(OH)ase deoxyribonucleic acid (DNA) and gene made it possible to screen for mutations. Vitamin D-dependent rickets type I (VDDR-1) occurs at high frequency in French Canadians; the disease locus in this population was mapped to chromosome 12q13-14 by linkage analysis. Renal 1α(OH)ase activity is regulated by PTH, calcitonin, calcium, phosphate, and $1,25(OH)_2D_3$ itself. By contrast, the recently cloned 1α(OH)ase in macrophages is not stimulated by PTH or calcitonin; however, 8-Br-cAMP and interferon (IFN)-γ increased the expression of the enzyme (90). FGF23 suppresses the renal enzyme 1α0(OH) hydroxylase. This is a key factor of

secondary hyperparathyroidism of CKD at all stages. The level of FGF23 is elevated already in every stage of CKD (53).

Osteomalacia in patients ingesting phenobarbital is associated with low levels of circulating $25(OH)D_3$. It has been shown that the biologic half-life of vitamin D_3 and $25(OH)D_3$ is shortened in phenobarbital-treated patients and that there is an accumulation of more polar metabolites, some of which are inactive (87). This rapid turnover and the production of inactive forms of vitamin D have been attributed to induction of microsomal enzyme activity in the liver. Phenytoin does not interfere directly with vitamin D metabolism but can induce hypocalcemia through reduced intestinal absorption of calcium and decreased release from bone. Low levels of circulating $25(OH)D_3$ also have been observed in patients with chronic hepatic failure (53,88).

Dietary calcium deprivation per se in rats increases the clearance and inactivation of $25(OH)D_3$ and leads to vitamin D deficiency. It has been suggested that this form of vitamin D deficiency is caused by secondary hyperparathyroidism, which increases the renal production of calcitriol. The latter augments the degradation of $25(OH)D_3$ to inactive metabolites. Hypothetically, this mechanism may account for vitamin D deficiency observed in clinical states of calcium malabsorption, including GI diseases, resection, or bypass; chronic liver disease; anticonvulsant therapy; and morbid obesity (53,91).

The clinical significance of abnormally low serum levels of $25(OH)_2D_3$ in patients with nephrotic syndrome owing to excessive urinary losses of vitamin D-binding globulin has not been fully established yet. However, a study of bone histology in patients with nephrotic syndrome who had normal renal function revealed that the decrease in $25(OH)D_3$ level results in a decrease in ionized calcium, secondary hyperparathyroidism, and enhanced bone resorption as well as defective mineralization. Interestingly, vitamin D has receptors in glomerular foot processes. It plays a protective role in preserving integrity and function of podocytes and other glomerular elements, via its specific calcium channel.

Our unpublished observations suggest that vitamin D supplementation may alleviate nephrotic syndrome and reduce the degree of proteinuria. It is well known that proteinuria leads to loss of vitamin D and precipitates significant vitamin D deficiency. It may require huge amounts of vitamin D to achieve full normalization of its levels in serum. Vitamin D level measurement is mandatory to avoid overdose (89).

Understanding of the metabolic pathways of vitamin D may facilitate

the investigation of various abnormalities. For example, low levels of $1,25(OH)_2D_3$ have been reported in patients with hypoparathyroidism. The lack of PTH and the presence of hyperphosphatemia may decrease the conversion of $25(OH)_2D_3$ to $1,25(OH)_2D_3$, which may explain the resistance to vitamin D in some patients with hypoparathyroidism. In support of this possibility is the finding of successful treatment of hypocalcemia with $1,25(OH)_2D_3$ in patients with hypoparathyroidism.

HYPERPARATHYROIDISM

Hyperparathyroidism is a common cause of hypocalcemia. Hyperparathyroidism can cause hypocalcemia with paresthesias, muscle spasms (i.e., tetany), and seizures, especially when it occurs rapidly. Chronic hyperparathyroidism generally causes hypocalcemia so gradually that the only symptoms may be visual impairment from cataracts following years of hyperparathyroidism. Hyperparathyroidism may be secondary or idiopathic.

SECONDARY HYPERPARATHYROIDISM

Hyperparathyroidism may be caused by surgery. This variety of hyperparathyroidism may result from accidental removal of parathyroids or traumatic interruption of their blood supply. Very often, the parathyroid deficiency is transient in nature. Hypocalcemia that appears after excision of parathyroid adenoma results from functional suppression and hypofunction of the remaining normal glands. Hyperparathyroidism may be a component of multiple endocrine dysfunctions, including adrenal insufficiency owing to an autoimmune disorder. In hyperparathyroidism associated with pernicious anemia, an autoimmune mechanism also has been implicated. Autoimmune hyperparathyroidism is commonly a part of polyglandular autoimmune syndrome type I (APS-1), which is familial syndrome. It occurs during childhood and is inherited as an autosomal recessive trait caused by mutations in an autoimmune regulator gene (AIRE). Loss of immunologic tolerance to tissue-restricted antigens due to the absence of AIRE expression in the thymus leads to exit of autoreactive T cells from the thymus that trigger autoimmunity (92). Antibodies against IFN-ω and -α have been recently shown to be a sensitive and specific marker for APS-1. APS-1 is associated with mucocutaneous candidiasis, vitiligo, and adrenal insufficiency. Antibodies against the CaSR in the parathyroid glands were found in many patients with this abnormality.

Adrenal insufficiency is a late phenomenon in this syndrome. Hyperparathyroidism is a recognized complication of thalassemia occurring after multiple transfusions and also has been described in patients with Wilson disease. Deposition of iron and copper in the parathyroid glands is the likely mechanism of parathyroid hypofunction in these patients (88). Also, parathyroid granulomas and metastatic cancer can lead to hypoparathyroidism.

Hypocalcemia may occur with magnesium depletion. Hypomagnesemia has been reported to induce skeletal resistance to PTH. It has been proposed that low serum magnesium diminishes the synthesis of PTH. It is interesting that some patients with hyperparathyroidism who exhibit resistance to vitamin D respond after administration of magnesium. Hypocalcemia associated with magnesium depletion responds poorly to intravenous calcium. Profound hypocalcemia may appear after therapeutic use of magnesium sulfate (e.g., in preeclampsia of pregnancy) because of suppression of PTH secretion. Certain drugs such as aminoglycosides and cytotoxic agents may have a direct toxic effect on parathyroid glands, leading to hypocalcemia. Irradiation of neck or administration of radioactive iodine also may affect parathyroid function (88). Symptomatic hyperparathyroidism also has been described in association with HIV infection.

IDIOPATHIC HYPOPARATHYROIDISM

Idiopathic hypoparathyroidism may be sporadic or familial. The familial congenital type is associated with hypocalcemic seizures in infancy. Familial idiopathic hypoparathyroidism is a heterogenous group of disorders. It may result from a mutation of the prepro-PTH gene or mutations of currently unidentified loci that affect the development, structure, or function of parathyroid glands (93,94).

The human PTH is encoded by a single gene that was mapped to the short arm of chromosome 11. Mutations in this gene may lead to familial hypoparathyroidism with autosomal dominant transmission. The levels of PTH in serum may be low or undetectable in affected patients (93,94).

The DiGeorge or velocardiaofacial syndrome consists of a congenital failure of development of derivatives of the third and fourth pharyngeal pouches, leading to absence of parathyroid glands and thymus. This syndrome may be inherited as an autosomal dominant disorder. It is associated with deletion of the long arm of chromosome 22 (95).

X-linked recessive hypoparathyroidism, like the DiGeorge syndrome,

is associated with parathyroid agenesis and undetectable PTH levels in circulation. The X-linked recessive hypoparathyroidism gene was mapped to the distal long arm of the X chromosome (93,94). Hypoparathyroidism may be caused by mutations or deletions in transcription factors or regulators of the development of parathyroid glands. Familial hypoparathyroidism due to dysgenesis of parathyroid glands results from mutations in the transcription factors glial cell missing B (GCMB) and glial cell missing 2 (GCM2). GCM2 is the master regulatory gene for parathyroid gland function and transcription factor for parathyroid gland development. A recent study has shown that one of the functions of GCM2 is to maintain high levels of CaSR expression in the parathyroid glands (96). Mutation in another transcription factor, globin transcription factor (GATA) protein-binding protein 3 (GATA3), which is a critical transcription factor for many developmental processes, causes an autosomal dominant complex disorder that combines hypoparathyroidism, deafness, and renal dysplasia.

A mutation in the gene coding for tubulin-specific chaperone E (TBCE), a peripheral membrane-associated tubulin-folding cofactor protein that is required for microtubule assembly, causes a rare autosomal recessive complex syndrome that includes hypoparathyroidism. The syndrome consists of hypoparathyroidism, retardation, and dysmorphism (HRD or Sanjad-Sakati syndrome). HRD is characterized by congenital hypoparathyroidism, intrauterine growth retardation, osteosclerosis, calcification of basal ganglia, mental retardation, seizures, and a typical facial dysmorphism featuring prominent forehead, deep-set eyes, and abnormal external ears. Mutation of the same gene was also reported in patients with autosomal recessive Kenny–Caffey syndrome (KCS). KCS resembles the HRD phenotype but is characterized by the presence of normal intelligence, late closure of the anterior fontanel, macrocephaly, postnatal growth retardation, and corneal opacity (97). Defects in maternal mitochondrial genes cause the Kearns–Sayre syndrome consisting of hypoparathyroidism, ophthalmoplegia, retinitis pigmentosa, cardiomyopathy with heart block, and diabetes mellitus.

Recently, autosomal dominant hypoparathyroidism was reported in families with activating mutations of the gene that encodes the extracellular CaSR in chromosome 3. In one family, missense mutation was found in the CaSR gene (98). These mutations cause excessive calcium-induced inhibition of PTH secretion. The hypocalcemia is mild and asymptomatic. It should be treated cautiously when mild because raising serum calcium concentrations markedly enhances urinary calcium

excretions, with increasing risk of nephrocalcinosis and renal insufficiency.

PSEUDOHYPOPARATHYROIDISM

Pseudohypoparathyroidism is a rare inheritable disorder characterized by mental retardation, moderate obesity, short stature, brachydactyly with short metacarpal and metatarsal bones, exostoses, radius curves, and expressionless face. The biochemical abnormalities are hypocalcemia and hyperphosphatemia (99,100). Some patients exhibit only the biochemical abnormalities. Thus, the disorder may be subdivided into pseudohypoparathyroidism type IA, which is also known as Albright hereditary osteodystrophy and IB.

Pseudohypoparathyroidism type IA is associated with both the somatic and biochemical abnormalities, and type IB, which presents with the biochemical defect, without the somatic abnormalities. In patients with pseudohypoparathyroidism, the administration of PTH fails to increase urinary cAMP and is not associated with phosphaturia. It has also been shown that the response to the administration of exogenous dibutyryl cAMP is intact in pseudohypoparathyroidism and causes pronounced phosphaturia. It has been proposed that the skeletal refractoriness to PTH shows a certain degree of selectivity. Accordingly, the bone responds to the remodeling action of the hormone but is resistant to its calcemic-homeostatic effect. Because of the hypocalcemic stimulus, secondary hyperparathyroidism may develop in some patients, leading to osteitis fibrosa cystica. Failure of the kidney to form $1,25(OH)_2D_3$ in response to PTH results in a low circulating level of this metabolite. This deficiency may be responsible, at least partly, for the skeletal refractoriness to the calcemic action of PTH that requires the presence of $1,25(OH)_2D_3$ (99).

Most patients with the type I form of pseudohypoparathyroidism manifest an approximately 50% reduction in cellular activity of the α-subunit of the G protein that stimulates adenylate cyclase ($G_s\alpha$) encoded by guanine nucleotide-binding protein α-subunit 1 ($GNAS_1$) gene. Patients with type IA show a generalized $G_s\alpha$ deficiency and often manifest resistance to other hormones whose effects are mediated by $G_s\alpha$-coupled receptors (e.g., calcitonins, glucagon, and thyroid-stimulating hormone). Pseudohypoparathyroidism type IA is caused by an inactivating mutation of the α-subunit of G_s and is inherited as an autosomal dominant trait. At variance with type IA, patients with pseudohypoparathyroidism type IB

manifest a selective end-organ resistance to PTH alone (100).

Other mechanisms have been identified in addition to the mechanism of target organ refractoriness. In one patient, the administration of PTH was associated with a normal increase in urinary cAMP but failed to produce phosphaturia (101). The latter is designated as pseudohypoparathyroidism type 2. Production of ineffective PTH, presumably because of a defect in the conversion of parathyroid prohormone into an active form, also was described in one patient (102). The patient had normal to high levels of immunoreactive PTH, which was probably a biologically inactive hormone, because the patient readily responded to exogenous PTH. Recently, a novel mutation of the signal peptide of the prepro-PTH gene has been described. This mutation leads to synthesis of defective PTH molecules and undetectable amounts of PTH in serum. This abnormality is inherited as an autosomal recessive type of isolated familial hypoparathyroidism (103).

Pseudo-pseudohypoparathyroidism occurs in families with pseudohypoparathyroidism type Ia. It presents inactivating mutations of $GNAS_1$ and features of Albright osteodystrophy but without the resistance to PTH and other hormones.

MALIGNANCY ASSOCIATED WITH HYPOCALCEMIA

Medullary carcinoma of the thyroid may present as a familial and autosomal dominant or a sporadic disorder. The tumor is derived from parafollicular cells of the ultimobranchial organ, which secrete calcitonin. Patients with this disorder have high levels of circulating calcitonin and exhibit an exaggerated increase in calcitonin in response to calcium infusion. Hypocalcemia has been reported to be present in some subjects. However, it is absent in others, and its absence despite very high levels of calcitonin is not well understood but has been attributed to a secondary increase in PTH. An “escape” from the effect of calcitonin, which has been observed in experimental conditions, is another possible factor. Elevated blood levels of calcitonin also have been reported in tumors other than medullary carcinoma of the thyroid, including carcinoma of the lung.

Hypocalcemia may develop in patients with malignant neoplasms in association with osteoblastic (bone-forming) metastases. The lesions may lead to rapid deposition of mineral in the newly formed matrix, thus causing hypocalcemia (104). Such hypocalcemia has been described in patients with carcinoma of the prostate or carcinoma of the breast with osteoblastic metastases (104). Although most of these patients have shown

osteoblastic lesions on radiologic examination, associated osteolytic lesions also have been present (104).

HYPERPHOSPHATEMIA

The various causes of hyperphosphatemia that may lead to hypocalcemia are listed in Table 6-3. The oral or intravenous administration of phosphate lowers serum calcium concentration in normal animals and hypercalcemic human subjects. This observation formed the basis for the clinical use of phosphate administration in states of hypercalcemia. The association of hyperphosphatemia and hypocalcemia has been reported to occur in a variety of circumstances. Hyperphosphatemia has been observed in persons ingesting large quantities of phosphate-containing laxatives or receiving enemas with phosphate. Hyperphosphatemia and hypocalcemia with tetany may develop in babies fed cow's milk, which contains 1,220 mg of calcium and 940 mg of phosphorus per liter (human milk contains 340 mg of calcium and 150 mg of phosphorus per liter).

The mechanism responsible for lowering serum calcium by the administration of phosphate is not entirely understood. One possibility is that the decrease in serum calcium concentration is caused by deposition of calcium phosphate in the bone, soft tissues, or both. The results of animal studies suggest that the administration of phosphate increases bone formation.

Table 6–3 Hyperphosphatemia as a Cause of Hypocalcemia

Administration of phosphate	Renal disease
Oral phosphate	Acute renal failure
Cow's milk in infants	Chronic renal failure
Laxatives containing phosphate	Neoplasms treated with cytotoxic agents
Potassium phosphate tablets	Lymphomas
Phosphate-containing enemas	Leukemias
Intravenous phosphate	Tumor lysis
	Rhabdomyolysis

In chronic renal failure, a constant increase in serum phosphorus concentration is observed when GFR is 30 mL/minute or less; and hyperphosphatemia is a common accompaniment of acute renal failure. It

is important to emphasize, however, that in renal failure causes other than hyperphosphatemia may play an important role in hypocalcemia. An acquired resistance to vitamin D, which might represent a metabolic block in the 1α-hydroxylation of 25(OH)D_3 to 1,25$(OH)_2D_3$ or skeletal resistance to the calcium-mobilizing effect of PTH, or both, is possibly involved. Recent clinical studies in human subjects suggest that impaired renal clearance of PTH may contribute to secondary hyperparathyroidism (105).

In patients undergoing chemotherapy for neoplastic diseases, particularly of lymphatic origin, large quantities of phosphates may be released into the circulation as a result of cytolysis. Spontaneous tumor lysis may cause hyperphosphatemia and consequently hypocalcemia. Conversely, rapid regrowth of tumoral masses may lead to profound hypophosphatemia (40).

ACUTE PANCREATITIS

The hypocalcemia associated with acute pancreatitis is not well understood. The precipitation of calcium soaps in the abdominal cavity, which results from the release of lipolytic enzymes and fat necrosis, has been suggested as the mechanism of hypocalcemia. Other studies implicate glucagon-induced hypersecretion of calcitonin as the mechanism of hypocalcemia in acute pancreatitis (88). These latter results have not been confirmed, however.

Even though it has been shown that hypocalcemia and urinary excretion of cAMP respond to pharmacologic doses of PTH, one study suggests a relative peripheral resistance to appropriate levels of endogenous hormone and normal circulating 1,25$(OH)_2D_3$. The cause of this refractoriness and its role in the hypocalcemia of acute pancreatitis are not apparent (106).

NEONATAL TETANY

Neonatal tetany with hypocalcemia was first described in 1913. Several mechanisms have been suggested for the pathogenesis of this disorder, namely vitamin D deficiency, parathyroid hypofunction, and hyperphosphatemia owing to a high content of phosphorus in the milk (cow’s milk).

Congenital absence of the parathyroid glands, usually in association with other congenital anomalies, has been reported in neonatal tetany.

Transient, idiopathic congenital hypoparathyroidism with hypoplasia or dysplasia of the parathyroid glands and with a subsequent compensatory hyperplasia has been described in infants with hypocalcemia (107). In one study, low levels of circulating immunoreactive PTH were detected in a group of babies with hypocalcemia. This finding was attributed to possible immaturity of the parathyroid glands, which was usually transient (108).

Babies born to mothers with osteomalacia caused by vitamin D deficiency have congenital rickets with hypocalcemia and tetany. In one study, serum levels of $25(OH)D_3$ were measured in 15 premature infants with neonatal hypocalcemia and their mothers. In 11 of the 15 cases, plasma $25(OH)D_3$ was low in both mother and infant (109). Babies born to mothers with hyperparathyroidism and hypercalcemia are at risk of hypocalcemia and tetany, probably because of suppression of the babies' own parathyroid glands.

OSTEOPETROSIS (MARBLE BONE DISEASE)

Osteopetrosis is a rare disease, with about 300 cases reported in the literature. The disease is characterized by abnormal bones that fracture easily, increased radiographic bone density, cranial nerve palsies because of compression of the nerves in their foramina, and mandibular osteomyelitis. There are two clinical forms of the disease. The first variant, malignant osteopetrosis, affects infants and usually is fatal. The second, benign osteopetrosis, may be recognized during any stage of adult life (110). The inheritance of the malignant form of the disease is recessive; inheritance of the benign form is autosomal dominant. Hypocalcemia has been found only in a few cases and does not appear to be a constant feature of the disease (110). The basic abnormality in osteopetrosis is not clear, but indirect evidence suggests that defect in osteoclast function leading to uncoupling between bone formation and resorption with reduced osteoclastic activity is the underlying mechanism.

The first physiologic defect described in human osteopetrosis was an autosomal recessive condition with lack of carbonic anhydrase II activity; 50% to 60% of children with severe osteopetrosis have mutation in gene proton pump H^+ ATPase. Osteoclasts in patients with proton pump defect are of normal appearance but dysfunctional. Mutations in chloride channel (CLCN7) appear less frequently as a cause of osteopetrosis (111).

Bone marrow transplantation and high doses of $1,25(OH)_2D_3$ with low calcium intake have been used in therapeutic trials. It has been

demonstrated that the vitamin D derivative, calcitriol ($1,25(OH)_2D_3$) may enhance the bone-resorbing activity of osteoclasts that is impaired in osteopetrosis (112). Recent trials of treatment with recombinant human IFN-*γ* are encouraging.

Sclerostin, discovered in 1999, is one of the most important hormones secreted by osteocytes. Sclerostin is a bone antianabolic peptide with negative bone regulation. Mutations of SOST gene, inactivating mutation have been associated with sclerosis. Sclerostin is an inhibitor of the Wnt/catenin signaling pathway expressed exclusively in mature osteocytes. When osteocytes are undergoing mechanical loading, sclerostin expression is suppressed. As alluded to, human machition mutation of sclerostin gene (SOST) leads to dense and strong bones called sclerosteosis. Antibodies to sclerostin are being developed for the treatment of osteoporosis. Sclerostin is increased at all levels of CKD. It has been speculated that sclerostin may play a role in the elevation of FGF23 in CKD (113).

ADMINISTRATION OF PHYTATE, SODIUM ETHYLENEDIAMINETETRAACETATE, CITRATE, AND MITHRAMYCIN

Sodium phytate (sodium inositol hexaphosphate) binds calcium in the intestine as calcium phytate and thereby inhibits calcium absorption. In normal subjects, the administration of phytate causes only a minimal drop in serum calcium, whereas it may precipitate hypocalcemia in patients with latent hypoparathyroidism. Excessive dietary phytate (cereals) has been implicated as a possible cause of osteomalacia in certain ethnic groups in England (91). Both citrate and sodium ethylenediaminetetraacetate (Na-EDTA), when given intravenously, bind ionized calcium and may produce hypocalcemia with low ionized calcium. Low serum-ionized calcium may be a complication of ethylene glycol (antifreeze) poisoning. This is because calcium binding by oxalic acid, which is the metabolite of the poison, reduces serum-ionized calcium.

Excessive intake of fluoride may induce hypocalcemia. This was reported recently in Alaska in connection with fluorosis that followed excessive addition of fluoride to drinking water (114). Drug-induced hypocalcemia was described in patients with acquired immunodeficiency syndrome. An analog of pyrophosphate, foscarnet, used to treat cytomegalovirus infection caused hypocalcemia because of chelation of calcium and concomitant hypomagnesemia (115). Ketoconazole and

pentamidine have been reported to cause hypocalcemia as well.

The association of low serum-ionized calcium with essential hypertension and secondary hyperparathyroidism has been described and attributed to renal calcium leak (116). This finding may be of clinical significance because a fall in serum-ionized calcium may compromise myocardial performance and worsen the function of a failing heart in patients with hypertension. Mithramycin is a potent inhibitor of RNA synthesis and has antitumor activity. It produces a decrease in serum calcium and phosphorus levels and in urinary hydroxyproline excretion. Mithramycin has been used to correct the hypercalcemia of various disorders, including malignancy with bone metastases. In experimental studies, mithramycin has been shown to inhibit the rate and magnitude of osteoclastic resorption induced by PTH; however, no demonstrable effect was found on normal bone formation or resorption in growing animals (117,118).

Hypocalcemia has been described recently in critically ill patients admitted to intensive care units. The incidence of hypocalcemia amounted to 88% in these patients. The degree of hypocalcemia correlated with the severity of the disease and was most commonly detected in patients who were septic. The mechanism of this abnormality is unknown. Circulating levels of calcitonin precursors (CTpr) increase up to several thousandfold in response to microbial infections, and this increase correlates with the severity of the infection and mortality. The relationship of elevated CTpr to the emergence of hypocalcemia needs to be investigated (119).

TREATMENT OF HYPOCALCEMIA

Symptomatic hypocalcemia generally responds promptly to the intravenous administration of calcium. The commonly used preparations are 10% calcium gluconate (10-mL ampoules containing 90 mg of elemental calcium) and 10% calcium chloride (10-mL ampoules containing 360 mg of elemental calcium). This treatment should be instituted immediately, because delay may be associated with further aggravation of tetany and lead to generalized seizures and even cardiac arrest.

Chronic treatment with oral calcium should follow the intravenous therapy in patients with chronic hypocalcemia owing to irreversible causes such as hypoparathyroidism. Oral calcium administration constitutes the best initial therapy in mild cases. The commonly used preparations are in tablet form: calcium lactate, 300 mg (60 mg of elemental calcium);

chewable calcium gluconate, 1 g (90 mg of elemental calcium); and calcium carbonate (Os-Cal), 250 mg of elemental calcium. Oral calcium also may be used for patients for whom the diagnosis of irreversible hypoparathyroidism has not been established with absolute certainty. In patients who fail to respond to oral calcium, vitamin D in large doses is the only available treatment. The commonly used preparations are capsules containing 1.25 mg (50,000 units) of vitamin D_2 (ergocalciferol). The average dose ranges between 1.25 and 3.75 mg/day. DHT_3 is three times as potent as vitamin D_2 in raising serum calcium concentration. Each capsule contains 0.125 mg of DHT_3. The average daily dose ranges between 0.25 and 1 mg of DHT_3. Both vitamins are available in liquid oil solutions as well. Both hypoparathyroidism and pseudohypoparathyroidism respond to physiologic doses of $1,25(OH)_2D_3$ and $\alpha(OH)D_3$ with restoration of serum calcium to normal. Calcitriol is marketed as Rocaltrol and is dispensed in capsules containing 0.25 and 1 μg. Chlorothiazides may enhance the calcemic action of vitamin D and its analogs, whereas furosemide may aggravate the hypocalcemia through its hypercalciuric action.

Patients in whom hypocalcemia is associated with hypomagnesemia respond poorly to intravenous calcium, but serum calcium concentration is restored to normal levels with correction of the hypomagnesemia.

Symptoms rarely develop in patients with chronic renal failure and hypocalcemia. However, very often, a reduction in elevated serum phosphorus with phosphate-binding antacids causes an increase in serum calcium concentration.

Hypocalcemia associated with osteomalacia resulting from vitamin D deficiency is rarely symptomatic. It usually responds to physiologic doses of vitamin D and increased oral calcium intake.

Disorders of Calcium and Phosphorus Metabolism Associated with Hypercalcemia

Hypercalcemia presents a challenge to every clinician and diagnostician. In some instances, the cause of hypercalcemia is self-evident on the basis of the circumstantial clinical findings, whereas extensive efforts are required to establish the etiology in other situations. The important causes of hypercalcemia are listed in Table 6-4.

HYPERPARATHYROIDISM

Primary hyperparathyroidism is present in 10% to 20% of all patients with hypercalcemia. The annual age-adjusted incidence is approximately 25 cases per 100,000. Making the diagnosis is very important because of this frequency and the amenability to surgical care. The disease is more common in females than in males; the incidence increases in women after menopause but is less frequent in older men. A single parathyroid adenoma is by far the most common cause of primary hyperparathyroidism. Carcinoma is very infrequent, occurring in <1% of all reported cases. Primary hyperplasia is found in <10% of all cases, but it is the most frequent cause in familial hyperparathyroidism.

Table 6–4 Disorders Associated with Hypercalcemia

Primary hyperparathyroidism	Hypervitaminosis D
Adenoma and carcinoma	Hypervitaminosis A
Hyperplasia	Granulomatous diseases
Multiple endocrine adenomatosis	Sarcoidosis
Ectopic secretion of PTH by neoplasms (rare)	Tuberculosis
	Histoplasmosis
Secondary hyperparathyroidism	Coccidioidomycosis
Malabsorption and vitamin D disease	Leprosy
Chronic renal failure	Foreign body granuloma
Following kidney transplantation	Hyperthyroidism
Familial hypocalciuric hypercalcemia	Adrenocortical insufficiency
Hypercalcemia associated with malignancy	Infantile hypercalcemia
Lytic bone metastases	Immobilization
Circulating tumor-secreted factors	Hypophosphatasia
PTHrP	Milk–alkali syndrome
1,25-Dihydroxyvitamin D_3-induced hypercalcemia	Parenteral nutrition
Locally acting, noncirculating, tumor-secreted cytokines	Hypercalcemia associated with acute renal failure
IL-1 and -6	Medications
TNF-β	Thiazides
Granulocyte macrophage	Lithium
Colony-stimulating factor	Theophylline

TGF-α	Calcium ion exchange resins
Prostaglandins	
Hypercalcemia in patients with hyperabsorptive hypercalciuria	

IL, interleukin; PTH, parathyroid hormone; PTHrP, parathyroid hormone–related peptide; TGF, transforming growth factor; TNF, tumor necrosis factor.

The morphologic differentiation between adenomas and hyperplasia sometimes is very difficult. The presence of a capsule and a rim of compressed normal gland tissue around the periphery of an adenoma may be helpful in making a definitive diagnosis. The persistence or recurrence of hypercalcemia after surgery for a purported adenoma warrants a more precise evaluation of the morphologic status of the parathyroid tissue removal. Also, with parathyroid hyperplasia, the quantity of parathyroid tissue to be removed—safely, yet not allowing recurrence of the disease on the other hand—is a very difficult balance to achieve. If more than one gland shows histologic features of hyperplasia, then removal of more than one gland is recommended; generally, approximately 200 mg of parathyroid tissue should remain.

In addition to the uncertainties related to morphologic differences between various forms of hyperparathyroidism, some of its functional characteristics have also been questioned. The widely accepted interpretation of the cause of the hypercalcemia in patients with parathyroid adenomas has been that the normal feedback regulation of PTH secretion is absent. That is, presumably the secreting cells of the adenoma were altered in such a way that their secretory function no longer responded to variation in serum calcium concentration; this state was defined as autonomy. The distinction between parathyroid adenoma and hyperplasia implied that the former is a primary disease rather than an adaptive response and that the latter represents a compensatory adaptation to low serum calcium concentration. The term “tertiary hyperparathyroidism” has been used to describe secondary hyperparathyroidism associated with an enormously enlarged mass of parathyroid tissue. Because of the inordinate number of secreting cells, large amounts of PTH enter the circulation despite the fact that each individual cell may respond normally to an elevation in serum calcium concentration by reducing the secretion of PTH from each cell. This is also supported by experimental studies (120). Some patients with primary hyperparathyroidism have pronounced hypercalciuria despite a very mild

degree of hypercalcemia and minimal or no bone disease (121). In patients with primary hyperparathyroidism, a very strong positive correlation was found between $1,25(OH)_2D_3$ in the serum and urinary calcium excretion. Patients with nephrolithiasis and hypercalciuria had higher circulating levels of $1,25(OH)_2D_3$ than those present in hyperparathyroid patients without renal stones (122). The reason for this difference in the $1,25(OH)_2D_3$ levels is unknown, but it stresses the importance of vitamin D metabolism in the clinical presentation of primary hyperparathyroidism.

New insights into additional factors that may predispose to hypercalciuria in patients with primary hyperparathyroidism have emerged recently. Polymorphism of CaSR in the presence of the R990G allele brings about gain of function of CaSR and results in increased susceptibility to hypercalciuria with consequent nephrolithiasis. It is likely that increased activation of CaSRs that reside at the basolateral surface of TALH triggers mechanisms that inhibit paracellular reabsorption of calcium causing hypercalciuria (9).

The high incidence of parathyroid adenomas in association with various malignant neoplasms is not well understood but warrants consideration in every case in which a malignant tumor is accompanied by hypercalcemia (123).

Molecular biology provides the means to study the role of genomic aberrations as the underlying mechanism of primary hyperparathyroidism. In parathyroid adenomas, changes were reported to occur in the gene that encodes PTH and is located on chromosome 11 (124). Likewise, alterations were identified in the X chromosome. The genomic abnormalities consist of loss of tumor-suppressor genes and/or overexpression of oncogenes on chromosome 11. Likewise, inactivation of tumor-suppressor genes was found in the X chromosome. It is interesting that these genomic changes were found not only in patients with parathyroid adenoma but also in patients with parathyroid hyperplasia, including hyperplasia secondary to chronic renal failure (125).

The familial occurrence of parathyroid adenomas with an autosomal dominant inheritance mandates the biochemical screening of family members of patients with primary hyperparathyroidism. Establishing the diagnosis of familial hyperparathyroidism also may be important to the patient’s surgeon, alerting him or her to the high incidence of hyperplasia and multiple adenomas in this group of patients. In some families, primary hyperparathyroidism is associated with other endocrine tumors as well. The syndrome of hyperparathyroidism, medullary carcinoma of the thyroid

with amyloid stroma, pheochromocytoma, and multiple neuromas is known as multiple endocrine neoplasia type II (MEN-II) or Sipple syndrome. The syndrome described by Wermer consisted of hyperparathyroidism and tumors of the pituitary and pancreatic islet cells (MEN-I).

MEN-I is an autosomal dominant familial neoplasia syndrome. The gene of MEN-1 ("menin") has been cloned. The gene was mapped to the long arm of chromosome 11. MEN-1 is a tumor-suppressor gene. Inactivating germ-line mutations of MEN-I gene lead to the growth of multiple endocrine neoplasia. Over 40 different germ-line mutations of MEN-I gene have been identified in MEN-I kindreds, suggesting the absence of a founder effect. By contrast, MEN-II is caused by activating mutation of the RET protooncogene and is inherited as an autosomal dominant trait. The hyperparathyroidism–jaw tumor syndrome consists of hyperparathyroidism cementoossifying fibromas of the jaw, renal cysts, Wilms tumor, and renal hamartomas. This syndrome is caused by a mutation of an unknown gene on chromosome lq24 and is inherited as an autosomal dominant trait.

A small minority of parathyroid adenomas have activating mutation of the cyclin D_1 oncogene ($CCND_1$). These mutations result in overexpression of the protein cyclin D_1 (126,127). It is interesting in this regard that primary hyperparathyroidism was induced by parathyroid-targeted overexpression of cyclin D_1 in transgenic mice.

Primary hyperparathyroidism can best be diagnosed by demonstrating persistent hypercalcemia with elevated serum PTH. Patients presenting with bone, renal, GI, or neuromuscular symptoms are considered symptomatic and usually require surgery. Conversely, in asymptomatic patients, objective manifestations of primary hyperparathyroidism that are indications for surgery include markedly elevated serum calcium concentration, a previous episode of life-threatening hypercalcemia, a reduced creatinine clearance, presence of kidney stones, hypercalciuria, and substantially reduced bone density (128).

Recent progress in imaging techniques includes the Tc 99m Sestamibi scan. This new technique helps detect and localize parathyroid adenomas with high precision and accuracy. Furthermore, this technique makes it possible to identify the adenoma intraoperatively with use of a portable radioactivity detector probe (Geiger counter) and guide the surgeon directly to the tumor. This advanced technique allows the surgical procedure to be carried out under local anesthesia with reduced morbidity

and with more successful outcome. Likewise, progress has been made with use of diagnostic ultrasonography. The close monitoring of PTH levels (PTH has a very short half-life during surgery) may assist in ascertaining the success of parathyroidectomy. A recent clinical study examined the clinical course and development of complications for a period up to 10 years in 121 patients with primary hyperparathyroidism; 101 (83%) of the patients were asymptomatic. During the study, 61 (50%) patients underwent parathyroidectomy, and 60 were followed up without surgery. Parathyroidectomy resulted in the normalization of biochemical values and increased bone mineral density (BMD). Most asymptomatic patients who did not undergo surgery did not have progression of disease; however, approximately one-fourth of them did have some progression. The progression included recurrent kidney stones, decrease of >l0% in bone density, rise to >12 mg/dL in serum calcium, and development of hypercalciuria. A recent study showed that in patients with asymptomatic hyperparathyroidism, parathyroidectomy improved the BMD as well as the quality of life. Parathyroidectomy has been recommended particularly for elderly patients with decreased BMD to prevent later fractures that carry high mortality. These findings raise serious questions regarding the choice of the optimal treatment for so-called “asymptomatic” patients with primary hyperparathyroidism (129).

Vitamin D status is one of the determinants of skeletal complications in patients with primary hyperparathyroidism. Low $25(OH)D_3$ and $1,25(OH)_2D_3$ levels are associated with increased turnover and decreased BMD in patients with primary hyperparathyroidism. This finding suggests that supplementation of vitamin D in vitamin D-deficient patients may be beneficial (130).

Familial hypocalciuric hypocalcemia is an unusual form of parathyroid hyperplasia with autosomal dominant transmission. There is a high incidence of neonatal primary hyperparathyroidism among the offspring of the affected families. The clinical course is relatively benign with an absence of nephrolithiasis and an infrequent occurrence of pancreatitis and chondrocalcinosis. Mild parathyroid hyperplasia with modestly elevated levels of circulating PTH and increased urinary excretion of cAMP have been reported in these patients. The unsatisfactory response to subtotal parathyroidectomy, however, suggests additional underlying abnormalities. The presence of hypocalciuria both before and after subtotal parathyroidectomy provides a strong argument that enhanced tubular reabsorption of calcium plays an important role in maintaining hypercalcemia. Hypermagnesemia, which appears to reflect increased

tubular reabsorption of magnesium, is another unique feature of this hypercalcemic disorder. It has been proposed that a concurrence of defects in both the parathyroid glands and kidneys in their response to serum calcium concentration is an explanation for this disorder (131).

Inactivating mutations in the human CaSR gene cause both FHH and neonatal severe hyperparathyroidism. The CaSR gene has been mapped to chromosome 3, the same chromosome to which the FHH disease locus was localized in the past. In most families with FHH, linkage to chromosome 3g predominates, although in one family linkage to chromosome 19f was demonstrated. Thus, the disease exhibits genetic heterogeneity. Inheritance of a single copy of mutated gene causes FHH, whereas homozygous patients who inherit two inactive genes develop neonatal severe hyperparathyroidism: The latter is associated with severe hypercalcemia owing to parathyroid hyperplasia that usually requires surgery. These mutations lead to a defective CaSR with a presumable impairment of signal transduction function, possibly resulting from abnormal coupling with G protein. This in turn appears to lead to abnormally reduced parathyroid and renal responsiveness to changes in extracellular calcium, resulting in increased PTH secretion and avid tubular reabsorption of calcium. Thus, the CaSR plays an important role in calcium-regulated secretion of PTH and tubular reabsorption of calcium. The FHH-associated excessive reabsorption of calcium, probably in the TAL, which persists even after parathyroidectomy, suggests that this abnormality is PTH independent (131,132).

Both familial and acquired forms of hypocalciuric hypercalcemia due to autoantibodies against CaSR have been reported. These autoantibodies inhibit receptor activation. Patients with the acquired form of autoimmune hyperparathyroidism presented with systemic autoimmune disease, including psoriasis, rheumatoid arthritis, hypophysitis with diabetes insipidus and hypothyroidism, uveitis and Coombs-positive anemia. The syndrome including hyperparathyroidism responded to immunosuppression with glucocorticoids, featuring a glucocorticoid-responsive hyperparathyroidism (133).

MALIGNANCY ASSOCIATED WITH HYPERCALCEMIA

A malignant neoplasm is the single most common cause of hypercalcemia. Hypercalcemia is most commonly produced by tumors of lung, breast, kidney, and ovary, and by hematologic malignancies. Very often, the hypercalcemia is uncontrollable and thus is a harbinger of the patient’s

demise. Indeed, survival after the appearance of hypercalcemia in association with malignancy is very poor, with a median of 3 months. Two main mechanisms are known to mediate the hypercalcemia of malignancy: local and humor. The local mechanism is manifested by the presence of osteolytic lesions in the skeleton. The malignant cells may act to destroy the bone directly; however, even local osteolysis is mediated by activated osteoclasts in most instances. Many tumors may produce hypercalcemia by a dual mechanism, that is, both local and humoral. It has been apparent that humoral hypercalcemia of malignancy (HHM) is caused by a circulating factor that is secreted by the neoplasm (134). This circulating substance acts on the bone to induce osteoclastic resorption and on the kidney to reduce phosphate reabsorption, increase calcium reabsorption, and increase nephrogenous cAMP excretion. All of these biochemical effects are characteristic of the actions of native PTH. However, only in four patients—one with small cell carcinoma of lung, the second with ovarian carcinoma, the third with metastatic neuroendocrine tumor of pancreas, and the fourth with hepatocellular carcinoma—was ectopic secretion of intact PTH demonstrated (135–137). In the vast majority of patients with HHM, the circulating factor is PTHrP. PTHrP is a 141 amino acid protein that binds to the receptors common to the native PTH, but it is encoded by a distinct gene. Even though PTHrP shares structural homology of *N*-terminal residues with PTH, immunoradiometric assay of PTH has been able to distinguish completely between patients with HHM and those with primary hyperparathyroidism (138,139). Thus, hypercalcemia with absence of detectable PTH by radioimmunoassay and presence of high urinary cAMP supports the diagnosis of HHM. PTHrP was originally isolated from human malignant tumors associated with HHM. Subsequently, it was detected in a variety of tissues, including parathyroid adenoma, skin, breast, placenta, testis, pancreas, and brain (139). With regard to the presence of PTHrP in parathyroid tissue, it has been suggested that PTH is produced by the chief cells (major component of parathyroid tissue), whereas PTHrP is produced by the oxyphil cells (139). Accordingly, the detection of PTHrP in circulation per se does not rule out parathyroid adenoma. Rather, the absence of PTH and presence of PTHrP by radioimmunoassays in fact rule out parathyroid adenoma and support the diagnosis of HHM.

In vitro PTHrP, similar to native PTH, has been shown not only to stimulate renal adenylate cyclase and increase the formation of cAMP, but also to activate 1α(OH)ase and enhance the formation of 1,25$(OH)_2D_3$. In vivo patients with HHM, however, contrary to patients with primary

hyperparathyroidism who may have high levels of serum $1,25(OH)_2D_3$, have low serum levels of calcitriol. In this regard, it has been reported that certain solid neoplasms produce substances that may inhibit the activity of lα(OH)ase and suppress the formation of $1,25(OH)_2D_3$. This appears to be the most tenable explanation for the low calcitriol levels in patients with HHM (140). The recently documented high circulating levels of FGF23 in malignancy provide a plausible explanation for the above discrepancy. FGF23 is a suppressant of 25(OH)d-1α(OH)ase and inhibits the production of $1,25(OH)_2D_3$ from its precursor $25(OH)D_3$. Another interesting feature that distinguishes between patients with primary hyperparathyroidism and those with HHM are the findings of bone histomorphometry. Whereas in patients with primary hyperparathyroidism bone resorption is closely matched with bone formation, in patients with HHM bone resorption and formation are uncoupled; specifically in HHM, enhancement of bone resorption and suppression of bone formation occur. The cause of this discrepancy is not readily apparent. Additional studies are necessary to determine whether malignancies produce factors that suppress bone formation (141).

CaSR is expressed in many malignant cells. Paradoxically, activation of CaSR by calcium increases the expression and secretion of PTHrP in cases of HHM, increasing osteolysis. In some cases, activation of CaSR promotes growth and spread of the tumor (142).

High PTHrP levels are present in 80% of patients with bone metastases from breast cancer who present with hypercalcemia, whereas PTHrP was present only in 12% of patients with breast cancer and metastases at sites other than bone. These findings are consistent with the notion that PTHrP may promote the development and growth of metastases in the bones by its potent osteolytic activity, which provides the environment for the proliferation of malignant cells (141). Hypercalcemia is a recognized complication of lymphoma, including both Hodgkin and non-Hodgkin types. Serum levels of $1,25(OH)_2D_3$ are either elevated or inadequately suppressed by the hypercalcemia in many patients with lymphoma-associated hypercalcemia. The elevated $1,25(OH)_2D_3$ levels may play a role in the pathogenesis of hypercalcemia. In some cases, chemotherapy induced normalization of serum calcium and a concomitant fall in $1,25(OH)_2D_3$. Conversely, reappearance of hypercalcemia was associated with recurrent rise above normal of $1,25(OH)_2D_3$ levels. Human T-lymphotropic virus-transformed lymphocytes are able to produce $1,25(OH)_2D_3$ from $25(OH)D_3$. Thus, there is a possibility that in some

cases of lymphoma, the malignant cells may have a similar capacity to produce $1,25(OH)_2D_3$, which may contribute to the development of hypercalcemia. However, it is noteworthy that the levels of PTHrP were elevated and considered to be responsible for the rise in serum calcium in a number of patients with lymphoma-associated hypercalcemia. Obviously, both PTHrP and elevated $1,25(OH)_2D_3$ may act synergistically to cause hypercalcemia (140).

HYPERCALCEMIA OF MALIGNANCY: THE ROLE OF OSTEOCLAST-ACTIVATING CYTOKINES

Tumor cells in bone and tumor-associated macrophages release factors that are known as osteoclast-activating cytokines. These tumor-derived factors, implicated in the development of hypercalcemia of malignancy, are IL-1, IL-6, tumor necrosis factor (TNF)-*α* (cachectin), TNF-*β*, lymphotoxin, transforming growth factor-*α* (TGF-*α*), and arachidonic acid metabolites. In addition, tumor cells may produce mediators (e.g., granulocyte macrophage colony-stimulating factor [M-CSF]) that induce immune cells to produce TNF and IL-1. Cytokines are produced and act locally as osteolytic factors. In most instances, the osteoclast-stimulating activity of the cytokines requires the presence of osteoblastic cells. Intravenous infusion of cytokines causes hypercalcemia in animals; however, these factors are believed to act locally in a paracrine fashion in clinical circumstances (141,143,144).

The role of osteoblastic stromal cells in tumor cell–induced osteolysis is depicted in Figure 6-6 (145). Tumor cells act indirectly by adapting to the physiologic mechanisms that promote bone resorption. Tumor cells release hormones (PTHrP), growth factors (TGF_2), cytokines (IL-6), and eicosanoids (prostaglandins), which act on osteoblastic cells to enhance the production of osteoclast-activating factors. Most important of these is the cell membrane–associated protein termed “RANKL,” a member of the TNF family of cytokines. RANKL can then bind to its cognate receptor (RANK) residing on the cell surface membrane of osteoclast precursors and in the presence of M-CSF promote the differentiation and fusion of the preosteoclasts to form active multinucleated osteoclasts. Concomitantly, production of soluble decoy receptors for RANKL termed “osteoprotegerin” (OPG) by osteoblastic cells inhibit osteoclastic osteolysis. Osteolytic bone matrix releases growth factors, including TGF-*β*, which accelerates tumor growth in the lysed area. Thus, a cycle is

activated where tumor cells and bone matrix interact to promote metastatic expansion. A recent study demonstrated that prostatic tumor cells may produce a soluble RANKL (sRANKL) and thus directly, without the mediating role of osteoblastic cells, accelerate osteoclastogenesis and osteolysis. In the same study, the administration of the decoy receptor for RANK, OPG, prevented the establishment of osseous tumors. These observations bear on possible nevi therapeutic avenues in preventing spread of prostatic tumor (145).

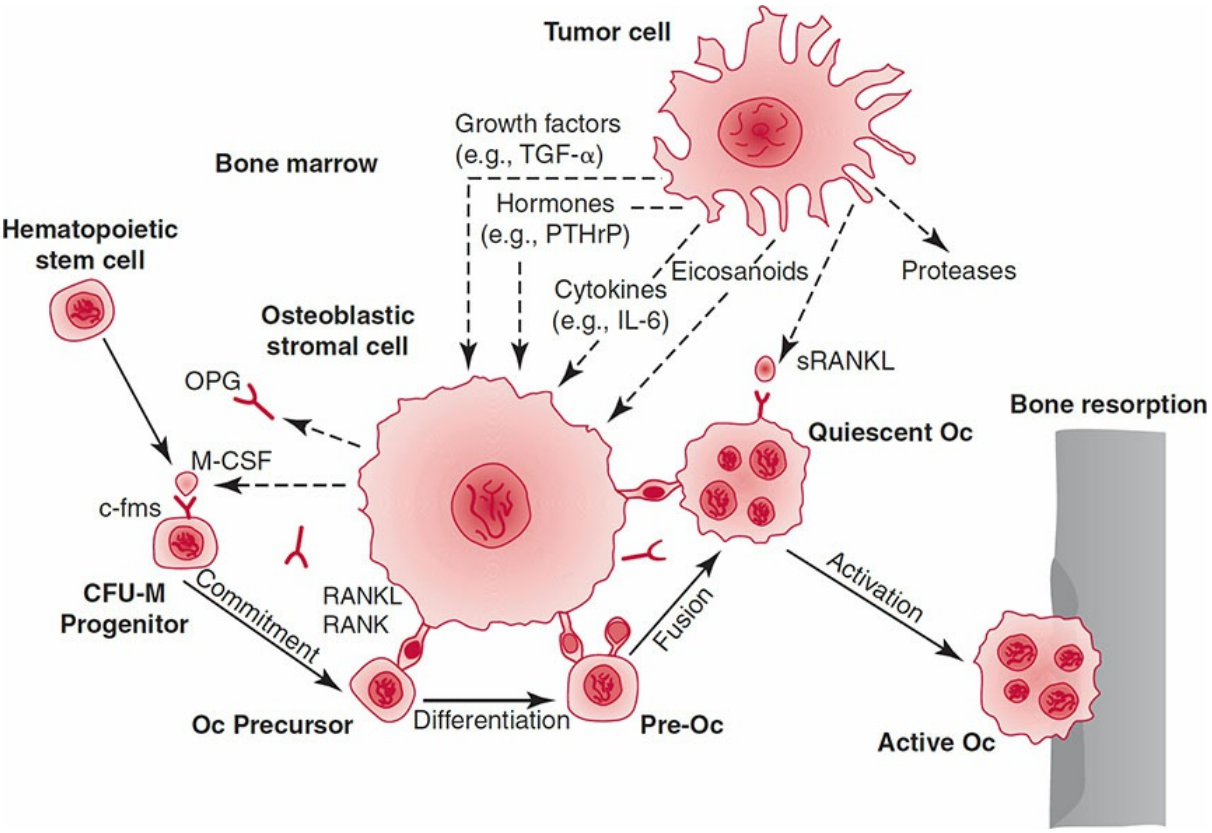


Figure 6–6 Schematic representation of tumor cell–induced osteolysis. A tumor cell may release soluble mediators such as hormones (e.g., PTHrP, eicosanoids), cytokines (e.g., IL-6), or growth factors (e.g., TGFα) that act on an osteoblastic stromal cell. The stromal cell produces RANKL, which binds to its cognate receptor, RANK, expressed on osteoclastic (Oc) precursors. In the presence of M-CSF, which acts on its receptor, c-fms, RANKL can enhance the formation of active osteoclasts that carry out bone resorption. Tumor cells also have been occasionally reported to directly release sRANKL, a soluble form of RANKL. Additionally, proteases can be produced by tumor cells and facilitate their invasion of nonmineralized tissue. (Republished with permission of American Society for Clinical Investigation from Goltzman D. Osteolysis and cancer. *J Clin Invest.* 2001;107:1219–1220; permission conveyed through Copyright Clearance Center, Inc.)

Hypercalcemia occurs in approximately one-third of patients with

myeloma. Osteolytic bone lesions are the most common skeletal radiographic findings. The bone destruction in myeloma is mediated by osteoclasts that accumulate adjacent to the collections of myeloma cells. This association of myeloma cells with osteoclasts in the past was believed to be related to the osteoclast-activating effect of cytokines that are locally secreted by malignant cells. Myeloma cells produce in vitro several osteoclast-activating factors, including TGF-β, IL-1, and IL-6. The increase in bone resorption in most cases is associated with a suppressed osteoblastic bone-forming activity. This explains the depressed skeletal uptake of bone-seeking radiolabeled elements in myeloma, resulting in negative bone scans in the vast majority of the affected patients. Myeloma cells exhibit a unique capability to grow rapidly in the bone. Myeloma cells secrete osteoclast-mobilizing stimulating cytokines, whereas osteoclasts secrete IL-6, which is a major growth factor of the myeloma cells. This relationship between myeloma cells and osteoclasts explains the rapid destruction of bone in this malignancy (141–147).

Additional information that has accumulated in recent years has shed new light on the molecular mechanisms activated by multiple myeloma cells on bone metabolism. Lytic bone destruction is the hallmark of myeloma. The osteolytic bone disease is mediated by osteoclasts. Previous research into the mechanism on myeloma addressed primarily the role of osteoclasts in uncoupling bone remodeling, tilting the balance toward resorption. Surprisingly, the factor that predisposes certain patients with myeloma to develop osteolytic lesions does not directly act on osteoclasts but rather on osteoblasts. Wnt (wingless/int) gene and its product protein are important factors in bone metabolism. The Wnt signaling pathway is important for the growth, differentiation, and maturation of osteoblasts. Interestingly, inactivating mutation of the gene for Wnt coreceptor, the low-density receptor-related protein 5(LRP5), causes an autosomal recessive disorder—the osteoporosis-pseudoglioma syndrome—with osteopenia and diminished osteoblast proliferation. Wnt signaling antagonist Dickopf 1 (DKKl) levels were found to be elevated in bone marrow plasma cells and peripheral blood from patients with myeloma who have osteolytic lesions. Only myeloma cells obtained from patients who had lytic lesions had detectable DKKl. It was not detected in normal cells, or those in myeloma patients with lytic lesions. It has been proposed that DKKl could block proliferation and differentiation of osteoblasts by blocking the canonical Wnt signaling. The mechanism by which DKKl can cause bone lesions in myeloma patients may involve two steps. Wnt promotes early proliferation of immature osteoblasts followed by the

differentiation into osteoblasts. DKK1 abrogates bone morphometric protein-2 (BMP-2)-mediated osteoblast differentiation into mature osteoblasts that build bone. Immature osteoblasts (osteoblast precursors) are rich sources of RANK ligand that plays a dominant role in inactivation and survival of osteoclasts. This leads to bone lytic lesions. On the other hand, reduced number and viability of mature functional osteoblasts downregulates bone formation and prevents filling the lytic lesions with new bone. This explains why the uptake of tracers with affinity to bone formation is absent in myeloma patients (148).

VITAMIN D INTOXICATION AND HYPERCALCEMIA

All patients receiving vitamin D, other than in small doses, for the treatment of hypoparathyroidism may develop hypercalcemia, with the attendant risk of renal failure. The appearance of hypercalcemia in hypoparathyroid patients receiving pharmacologic doses of either ergocalciferol (vitamin D_2) or DHT_3 is almost unpredictable, because the margin between normocalcemic and hypercalcemic doses of the vitamin is very narrow. Some episodes of hypercalcemia may pass unnoticed and yet be the underlying cause of reduced renal function in these patients. The administration of thiazide diuretics also may be an aggravating factor in this situation, partly because it reduces the urinary excretion of calcium. The hypercalcemia associated with vitamin D intoxication may be present from 1 to 6 weeks after discontinuation of the treatment, and the normocalcemia may persist for an additional 4 months without any treatment. The toxic effect of vitamin D excess is associated with a high level of circulating $25(OH)D_3$, which is continuously produced by the liver from the adipose tissue stores of vitamin D. The serum level of $1,25(OH)_2D_3$ generally is not elevated and even may be reduced (149). The hypercalcemia associated with $1,25(OH)_2D_3$ administration, however, is much more short-lived (3–7 days).

Various factors may alter the response to vitamin D. The inhibitory effect of estrogens on bone resorption may be absent after menopause, which allows more calcium to be released from the bone for any given dose of vitamin D. The administration of corticosteroids may reduce the effect of vitamin D; in fact, corticosteroids may be used to treat vitamin D intoxication. The most important precaution in preventing the complications of vitamin D intoxication is to measure serum calcium concentrations frequently in these patients. Likewise, the presence of

excessive hypercalciuria, even in the absence of hypercalcemia, is a risk factor for nephrocalcinosis and renal failure. Thus, monitoring of urinary calcium excretion in these circumstances is recommended as well.

VITAMIN A INTOXICATION AND HYPERCALCEMIA

Hypercalcemia associated with vitamin A intoxication has been much discussed (150,151). This condition has been associated with excessive intake of vitamin A, which is readily available for sale in various pharmaceutic preparations (151). Isotretinoin, a derivative of vitamin A that is effective in the treatment of severe nodulocystic acne, has been reported as a cause of hypercalcemia (152,153). The main symptom of vitamin A intoxication is painful swelling over the extremities. Prolonged hypercalcemia in this condition also has been associated with nephrocalcinosis and impairment of renal functions (150). In experiment animals, excessive amounts of vitamin A cause fractures, increased number of osteoclasts, and calcification of soft tissues. In human subjects, periosteal bone deposition constitutes the typical radiographic feature (154).

SARCOIDOSIS AND HYPERCALCEMIA

Hypercalcemia in patients with sarcoidosis is associated with increased intestinal absorption of calcium and increased calcium release from the bone; it is found in about 17% of all patients with sarcoidosis (155). It is more frequent in males than in females (126). In a small proportion of patients, very high serum calcium concentration leads to metastatic calcifications and eventual death owing to uremia (156). The hypercalcemia may disappear with the appearance of uremia (157).

Seasonal incidence of hypercalcemia in sarcoidosis is directly related to the amount of sunlight exposure (157). Plasma levels of $1,25(OH)_2D_3$ have been found to be increased in patients with sarcoidosis and hypercalcemia, a finding that accounts for the abnormal calcium metabolism in this disease (158). In most of the patients, hypercalcemia may be corrected with the administration of glucocorticoids, which restores to normal both the elevated calcium and $1,25(OH)_2D_3$ concentrations in the serum (159,160). Serum immunoreactive PTH has been found to be low in patients with sarcoidosis, regardless of the presence or absence of hypercalcemia.

In vitro studies demonstrated the production of $1,25(OH)_2D_3$ by

primary cultures of pulmonary alveolar macrophages harvested from patients with active sarcoidosis (161). Thus, the pathogenesis of hypercalcemia in sarcoidosis is extrarenal production of $1,25(OH)_2D_3$ by the macrophage, which is a major constituent of the sarcoid granuloma. A similar mechanism appears to be responsible for the hypercalcemia associated with other granulomatous diseases. Hypercalcemia has been reported in tuberculosis, leprosy, foreign body–induced granuloma, silicone-induced granuloma, disseminated candidiasis and coccidioidomycosis, histoplasmosis, berylliosis, granulomatous lipoid pneumonia, and eosinophilic granuloma (157–160). Whereas $1,25(OH)_2D$ synthesis in renal mitochondria by 25(OH)D-1α(OH)ase from 25(OH)D is a regulated process, extrarenal synthesis is not well regulated. Recent research results provide evidence that activation of toll-like receptors (TLRs) by microbial lipopolysaccharide results in upregulation of 25(OH)D-1α(OH)ase in macrophages. The local production of $1,25(OH)_2D_3$ induces the expression of an antimicrobial peptide cathelicidin, which is considered to be a key factor in the innate immune response. When TLR is activated by an infective agent such as *Mycobacterium tuberculosis*, it produces the antimicrobial factor. This observation can be used to explain the possible beneficial effect of vitamin D induced by exposure to sunlight at high altitudes in patients with tuberculosis.

HYPERTHYROIDISM, HYPOTHYROIDISM, AND HYPERCALCEMIA

The incidence of hypercalcemia in patients with hyperthyroidism varies from 10% to 22% in different reports (162). This hypocalcemia may be reversed by antithyroid therapy. Because the association of hyperthyroidism and hyperparathyroidism has been reported to be common, the therapeutic response of the hypercalcemia to the antithyroid therapy may be of some diagnostic significance (162). The effect of thyroid hormone on calcium metabolism primarily consists of increased bone turnover, increased urinary calcium excretion, and decreased intestinal absorption of calcium, with a resultant negative calcium balance (163). Thus, the action of thyroid hormone on bone is primarily responsible for the hypercalcemia. Thyroid hormone enhances the ability of PTH to increase bone reabsorption and directly enhances bone resorption in vivo in the absence of PTH (164). Serum phosphate may be

elevated in hyperthyroidism, possibly because of suppression of parathyroid activity by the hypercalcemia and subsequent enhanced tubular reabsorption of phosphate.

Serum calcium and phosphate levels are normal and alkaline phosphates are low in the vast majority of patients with hypothyroidism; however, some patients may manifest hypercalcemia. Calcium balance in patients with hypothyroidism tends to be positive as a result of increased intestinal absorption and reduced urinary excretion. Both changes predispose to the development of hypercalcemia. The bone turnover in hypothyroid patients is reduced.

ADRENAL INSUFFICIENCY AND HYPERCALCEMIA

Hypercalcemia is a common abnormality in adrenal insufficiency (165,166). The mechanism of hypercalcemia in this clinical setting is not well understood. One study indicates that the increase in serum calcium concentration is caused by an increase in the protein-bound fraction of serum calcium that results from accompanying volume depletion. The volume depletion also may cause an increase in the renal tubular reabsorption of calcium, and vitamin D's enhancement of calcium absorption from the intestine may be greater in the absence of glucocorticoid hormone.

IDIOPATHIC INFANTILE HYPERCALCEMIA

Idiopathic infantile hypercalcemia encompasses a group of disorders characterized by hypercalcemia during infancy, mostly of a transient nature. It can be divided into benign and severe types according to the gravity of the clinical manifestation. The benign type is associated with minimal symptomatology and has an excellent prognosis. The severe form is associated with serious somatic sequelae including mental deficiency, "elfin" face with depressed nasal bridge, epicanthal folds, supravalvular aortic stenosis, bladder diverticula, degenerative renal disease, occasionally pulmonic stenosis, ventricular septal defects, and dental abnormalities. These somatic distortions, known as Williams syndrome, were believed to reflect developmental defects resulting from hypercalcemia, probably already present in the fetal stage. The hypercalcemia is of limited duration; however, the somatic abnormalities are permanent. Thus, many patients suffering from Williams syndrome who present with the clinical syndrome fail to show abnormalities in

calcium metabolism. The primary genetic abnormality is deletion of one allele of the elastin gene. Hemizygosity for this gene was detected in 75% of patients. This defect is probably responsible for the vascular, valvular, and developmental defects.

Idiopathic infantile hypercalcemia has been attributed to hypersensitivity to vitamin D. In support of this possibility is the finding that hypercalcemia in this syndrome may occur with small doses of vitamin D, which are only two to three times larger than the physiologic dose (167). The high incidence of this syndrome in a group of infants in England who were drinking milk fortified with excessive amounts of vitamin D, and its disappearance when vitamin D was eliminated from the diet, supported the possibility that the syndrome resulted from hypersensitivity to vitamin D (168,169).

However, there is no unifying pathogenesis underlying the abnormal calcium metabolism in idiopathic infantile hypercalcemia. Increased serum levels of $1,25(OH)_2D_3$ have been considered to be the mechanism of hypocalcemia by some investigators (167,168). Others have failed to show that abnormality even in the presence of hypocalcemia. Abnormalities in the regulation of calcitonin secretion with reduced stimulation by hypocalcemia were advanced as the possible mechanism by others.

Deletion of approximately 25 to 30 genes spanning about 1.5 megabases in the q11.23 region on chromosome 7 has been identified in some patients with this syndrome. Attention has been recently focused on deletion of the so-called Williams syndrome transcription factor (WSTF). The role of WSTF gene deletion in the alleged hypersensitivity to vitamin D is unknown.

It has been proposed that human multiprotein complex (WINAC) that mediates recruitment of unligated VDR to target sites in promoters acts via the WSTF (170,171). Recently, evidence has been advanced that WSTF has an intrinsic tyrosine kinase activity that is involved in chromatin remodeling in response to DNA damage. It is possible that the ATP-dependent chromatin complex that incorporates the WSTF potentiates ligand-induced VDR action in both gene transactivation and repression. In the latter case, WSTF gene deletion would remove the repression and lead to enhanced response to vitamin D–VDR complex. All the above hypotheses are conjectural at this time (172).

Hypocalcemia with fat necrosis is a peculiar variant of the disease. In this syndrome affecting infants, only hypocalcemia occurs, with areas of necrosis of subcutaneous fat tissue (168). In some cases, high levels of $1,25(OH)_2D_3$ were reported. Some investigators maintain that

hypocalcemia is not a primary but rather a secondary phenomenon. In the latter instance, it has been proposed that the rise in $1,25(OH)_2D_3$ leading to hypercalcemia is secondary to the granulomatous inflammation of the fat necrosis. Irrespective of the mechanism, idiopathic infantile hypocalcemia is treated by dietary restriction of calcium and vitamin D.

IMMOBILIZATION AND HYPERCALCEMIA

Immobilization may be associated with excessive loss of bone minerals, hypercalcemia, and rapidly developing osteoporosis. The lack of postural mechanical stimuli to the skeleton disturbs the balance between bone formation and reabsorption, thus leading to loss of bone mass and its minerals. Usually, the amount of calcium released from the bone is excreted in the urine and does not increase the serum calcium concentration (173). However, in states of rapid bone turnover, which are present in normal children and adolescents and in patients with bone abnormalities such as Paget disease, immobilization may result in overt hypercalcemia.

HYPOPHOSPHATASIA

Hypophosphatasia is a syndrome characterized by low serum alkaline phosphatase, high serum levels of pyrophosphate, and skeletal abnormalities resembling osteomalacia (174). The disorder may be associated with hypercalcemia, especially in infants.

MILK–ALKALI SYNDROME

Milk–alkali syndrome ranks third, after primary hyperparathyroidism and malignancy, as the most common cause of hypercalcemia. It occurs in patients who ingest large amounts of milk and alkali as a therapy to relieve the symptoms of peptic ulcers. Likewise, the recommended consumption of calcium carbonate for prevention and treatment of osteoporosis has increased the frequency of this iatrogenic hypercalcemia. The syndrome is characterized by hypercalcemia, hyperphosphatemia, alkalosis, metastatic calcifications, and progressive renal failure. It has been shown that these abnormalities may be reversed by discontinuation of the therapy. Large doses of calcium carbonate seem to be the major factor in the development of this syndrome, because the use of antacids other than calcium carbonate does not lead to hypercalcemia (175). Therefore, it appears that the

hypercalcemia of milk–alkali syndrome results from high oral loads of calcium carbonate and causes renal retention of phosphate by suppressing PTH secretion. The resulting serum calcium–phosphorus product leads to metastatic calcification and impairment of renal function. The increased activation by high serum calcium of CaSR at the basolateral surface of loop of Henle cells enhances natriuresis and induces volume depletion that augments proximal reabsorption of calcium. The attendant alkalosis activates the pH-sensitive calcium channel, TRPV5, in the distal nephron, thereby contributing to calcium retention and hypercalcemia. Increased oral intake of calcium carbonate has also been reported to induce hypercalcemia in uremic patients. Similarly, the use of calcium-containing exchange resins for the treatment of hyperkalemia may cause hypercalcemia because of the release of calcium from the resin in the intestinal lumen (176).

Hypercalcemia has been described in patients recovering from acute renal failure. The etiology is not well understood, but in some patients it may result from the combination of secondary hyperparathyroidism and released calcium from traumatized, necrotic muscle (177,178) and from high calcitriol levels produced by the traumatized muscles.

THIAZIDE DIURETICS AND HYPERCALCEMIA

Chronic administration of thiazide diuretics may lead to hypercalcemia in patients treated with large doses of vitamin D (hypoparathyroid patients and patients with osteoporosis) and in patients with hyperparathyroidism. The mechanism of action may involve: (a) reduced urinary excretion of calcium resulting from a direct tubular effect, or ECF depletion with secondary increase in tubular reabsorption of sodium and calcium, or both; and (b) increased bone responsiveness to the resorptive actions of vitamin D and PTH (178,179). Thiazide-induced inhibition of apical entry of sodium lowers cytosolic sodium concentration. The latter leads to a steeper gradient between intracellular and peritubular sodium concentrations. An increased sodium gradient would favor pumping more calcium out of the cell via NCX, enhancing calcium reabsorption. It has been demonstrated that thiazides may acutely enhance the hypercalcemic skeletal response to PTH in the absence of changes in ECF volume. Recent studies suggest that primary hyperparathyroidism is common in patients who develop hypercalcemia while taking thiazide diuretics. Therefore, it is likely that thiazides “uncover” mild primary hyperparathyroidism in many patients. The above notion is supported by previous studies that demonstrated that

the calcemic effect of thiazides is PTH dependent (180). A recent study demonstrated expression of differentiation and bone formation (170).

LITHIUM AND THEOPHYLLINE

Patients treated chronically with lithium may develop hypercalcemia with elevated PTH levels. In this regard, primary hyperparathyroidism and hypothyroidism have been reported in patients treated with lithium. Theophylline toxicity also may be associated with hypercalcemia, probably because of stimulation of β-receptors in the bone.

TREATMENT OF HYPERCALCEMIA

Lowering of serum calcium concentration can be produced by: (a) inhibiting calcium release from the bone, or increasing its deposition in the bone and other tissues, or both; (b) increasing removal of calcium from the ECF or inhibiting its absorption in the bowel; and (c) decreasing the ionized fraction by complex formation with chelating substances.

Hypercalcemia augments urinary losses of sodium and water, resulting in the contraction of extracellular volume and reduced GFR. The latter leads to diminished urinary excretion of calcium and further aggravation of hypercalcemia. Therefore, the first therapeutic goal is to restore the extracellular volume to normal by intravenous administration of normal saline. This usually requires 3 to 4 L of saline. This therapeutic action per se lowers the serum calcium concentration, partly by the dilutional effect and partly by increased urinary excretion of calcium. There is a risk of extracellular volume overload during a rapid intravenous administration of saline, which is particularly hazardous in elderly patients. Therefore, monitoring of central venous pressure in this situation may be very helpful. Likewise, the addition of loop diuretics as an adjunct therapy may not only minimize the risk of fluid overload but also substantially increase the urinary excretion of calcium. The effect of loop diuretics as calciuretic agents requires prompt replacement of urinary losses of sodium and water. The use of loop diuretics may be particularly beneficial in patients who develop hypercalcemia as a result of excessive secretion and high serum level of PTH, PTHrP, or both. Hormone-induced excessive tubular reabsorption of calcium plays a major role in the development and maintenance of hypercalcemia in these circumstances.

Bisphosphonates

Bisphosphonates (formerly diphosphonates) represent a group of drugs with a high therapeutic potential for the treatment of hypercalcemia in general and that associated with malignancy in particular. Bisphosphonates are related to an endogenous product of bone metabolism, pyrophosphate. The P-O-P bonds of pyrophosphate are cleaved by phosphatase in the process of bone mineralization and osteoclastic bone resorption. In the bisphosphonates, carbon replaces the oxygen moiety, generating a P-C-P bond, which is resistant to hydrolysis by phosphatase. Bisphosphonates have a great affinity for bone and bind tightly to calcified bone matrix, impairing both the mineralization and resorption of bone. In addition, they interfere with the function of osteoclasts. They appear to have several direct effects on osteoclast function, including prevention of osteoclast attachment to bone matrix and prevention of osteoclast differentiation and recruitment. Bisphosphonates also inhibit the motility of isolated osteoclasts. Thus, they are very potent inhibitors of bone resorption. The first bisphosphonate, ethane-hydroxybisphosphonate (etidronate; Didronel), is now available for clinical use, but its potency as an antihypercalcemic agent is limited, at least when given orally. Probably, this is because its effect to reduce bone resorption is offset by its effect to inhibit bone mineralization. Reduction in serum calcium concentration has been achieved more successfully with the second generation of bisphosphonates, including dichloromethylene bisphosphonate (clodronate) and amino-hydroxypropylidene bisphosphonate (pamidronate; ADPC), which causes a reduction in bone resorption with a dose that has a negligible effect on bone mineralization. Pamidronate and eitdronate currently are approved for treatment of hypercalcemia of malignancy in the United States. In clinical trials, pamidronate and clodronate have been demonstrated to inhibit hypercalcemia, bone pain, and pathologic fractures in patients with malignancy-associated hypercalcemia. Pamidronate is most effective when given intravenously; a single infusion of 30 mg achieved normocalcemia in 90% of patients in one study. A comparison shows that the effect of 30 mg of pamidronate is equal to 600 mg of clodronate and 1,500 mg of etidronate in controlling hypercalcemia. The third generation of bisphosphonates, including alendronate, risedronate, and tiludronate, in preliminary studies is 500 times more efficient in inhibiting bone resorption than clodronate. Zoledronic acid is a new generation of nitrogen-containing bisphosphonate that in clinical studies was superior to pamidronate. This agent has been approved for clinical use.

The molecular mechanisms underlying the pharmacologic

antiosteoclast action differs between the two major classes of bisphosphonate: (1) non–nitrogen-containing bisphosphonates (etidronate and clodronate) and (2) the more potent, nitrogen-containing bisphosphonates (pamidronate, alendronate, ibandronate, risedronate, and zoledronate). The first class bisphosphonates bind with nonhydrolyzable analogs of ATP that accumulate in osteoclasts and block mitochondrial energy production, leading to osteoclast apoptosis. By contrast, the nitrogen-containing class of bisphosphonates inhibit farnesyl pyrophosphate synthase, thereby blocking prenylation of small GTPase proteins that are required for normal function and survival of osteoclasts.

Glucocorticoids

Glucocorticoids are effective in lowering serum calcium in states of vitamin D intoxication, sarcoidosis, and malignancy. The exact mode of their action is not well understood, but the possible mechanisms are suppression of bone resorption and decreased intestinal absorption. It has been pointed out that glucocorticoids are more effective in hypercalcemia associated with lymphoma, leukemia, and multiple myeloma than with other neoplasms. This effect of glucocorticoids might be related to a tumor lytic effect, interference with the production of osteoclast-activating cytokines, or both. The average effective dose is 3 to 4 mg/kg/d of hydrocortisone given intravenously or orally. The fall in serum calcium concentration occurs 1 to 2 days after starting the therapy.

Calcitonin

Calcitonin lowers serum calcium concentration by inhibiting bone resorption and by increasing urinary calcium excretion. The administration of calcitonin is associated with negligible toxicity; however, its therapeutic action has a limited duration because of the osteoclast escape phenomenon, which is apparent several days after starting therapy. Addition of glucocorticoids may be helpful to maintain efficacy.

Mithramycin (Plicamycin)

Mithramycin is a cytotoxic substance derived from an actinomycete of the genus *Streptomyces* and is used mainly in the treatment of testicular tumors. Mithramycin lowers serum calcium concentration by suppressing bone resorption. The dose, which is lower than the antitumor dose and has

fewer side effects, is 25 μg/kg given intravenously. The drug is available commercially as Mithracin. The effect starts 24 to 48 hours after injection and lasts several days. Side effects are suppression of bone marrow activity and hepatocellular and renal toxicity, which usually occur with repeated doses.

Phosphate

Oral and intravenous salts of phosphorus lower serum calcium concentration and reduce urinary excretion of calcium. This effect has been variously attributed to: (a) deposition of mineral in the bone; (b) increased deposition of calcium in soft tissue; and (c) suppression of bone resorption. The major untoward side effects of this therapy are extraskeletal calcifications, including nephrocalcinosis with resulting renal failure. Thus, the use of phosphates to treat hypercalcemia should be discouraged in patients with high serum phosphates and renal insufficiency. Phosphates may be given intravenously at a dose of 20 to 30 mg of elemental phosphorus per kilogram of body weight over 12 to 16 hours. Serum calcium concentration should be determined at close intervals. The commercially available preparation for intravenous use is InPhos; 40 mL of the solution contains 1,000 mg of phosphorus, 65 mEq of sodium, and 8 mEq of potassium.

Other Therapies

Gallium nitrate has been approved by the U.S. Food and Drug Administration (FDA) for therapy of hypercalcemia. It inhibits bone resorption by reducing the solubility of hydroxyapatite crystals. Nephrotoxicity is a major side effect of gallium nitrate. The use of somatostatin congener (lanreotide) has been reported to successfully inhibit hypercalcemia in a patient with a PTHrP-secreting pancreatic neoplasm. The calcium-lowering effect was associated with suppression of the serum levels of PTHrP (180). The hypercalcemia associated with thyrotoxicosis and theophylline toxicity has been successfully treated with intravenous propranolol. Denosumab is a human monoclonal antibody against RANKL. It works by preventing the development of osteoclasts, the bone-resorbing cells. It has been approved by the FDA for the treatment of hypercalcemia of malignancy that is resistant to the action of bisphosphonates. It has also been approved for the treatment of postmenopausal osteoporosis (181).

Intestinal absorption of calcium may be reduced by dietary restrictions and binding of calcium in the bowel with cellulose phosphate and sodium phytate to form nonabsorbable complexes. Calcium also may be removed directly from the ECF with hemodialysis or peritoneal dialysis by employing calcium-free dialysate solution. Reduction in serum-ionized calcium may be accomplished with intravenous Na-EDTA, which is a chelating agent. The complexed calcium then is excreted in the urine. The main disadvantage of this therapy is the nephrotoxicity of EDTA.

Metabolic Bone Diseases

RICKETS AND OSTEOMALACIA

Rickets and osteomalacia are metabolic disorders of the bone in which the mineralization process of the epiphyseal cartilage and organic bone matrix is impaired. This abnormality results in a decreased amount of mineralized bone (Fig. 6-7) and an increased amount of osteoid (or cartilage), which cause decreased mechanical strength of the bones. Therefore, the bones become soft, bend easily, and are liable to deformities and pseudofractures (Fig. 6-8). It should be mentioned that such an increase in the width of osteoid seams may be seen in conditions other than osteomalacia. The bone formation is rapid in Paget disease, and there may be a lag between the rate of apposition of bone matrix and its mineralization. Therefore, this sequence leads to an increased width of osteoid seams. Histologically, however, the presence of a calcification front in bones from patients with Paget disease and its absence in osteomalacia allow the distinction between these diseases. The calcification front may be demonstrated by specific histochemical staining techniques or the administration of a tetracycline, which is incorporated specifically in the calcification front. The calcification front reappears during the healing of osteomalacia.

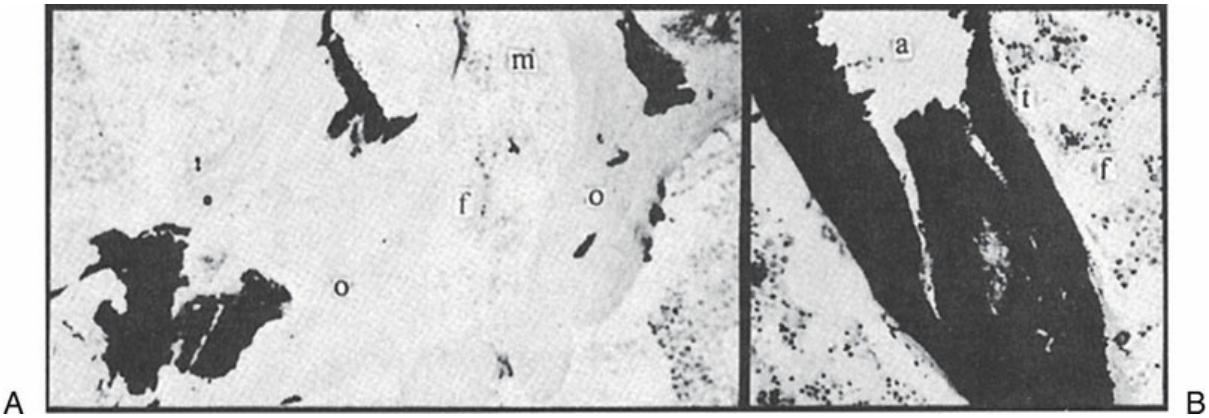


Figure 6–7 (**A**) Osteomalacia. Bone trabeculae are calcified (*black stain*) only in the center, with a wide rim of osteoid tissue. (**B**) Normal bone. All bone trabeculae are calcified (*black stain*). (*a*), artifact; (*f*), fat; (*m*), marrow; (*o*), osteoid; (*t*), trabeculae.

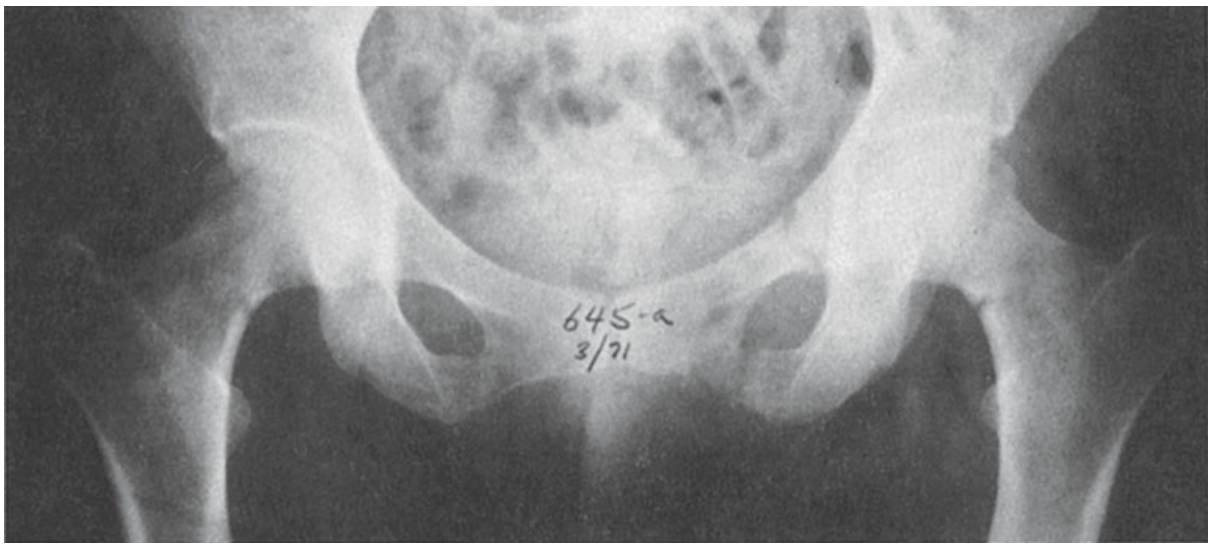


Figure 6–8 Roentgenographic appearance of osteomalacia. The radiolucent lines on the necks of both femurs are pseudofractures.

The major symptoms of osteomalacia are diffuse bone and muscle pains, which cause disability and increasing needs for analgesic medication. The etiologies of osteomalacia (Table 6-5) can be divided into two principal subgroups. The first group, which is the most common, is associated with abnormally low serum concentrations of phosphorus and calcium. In the second group, which is less common, the defect in mineralization is related in some way to abnormalities in the organic matrix and is not associated with a low serum calcium–phosphorus product.

Vitamin D deficiency causes osteomalacia primarily by decreasing the absorption of calcium and phosphorus from the intestine and thereby decreasing the serum concentrations of calcium and phosphorus. As

mentioned, osteomalacia caused by vitamin D deficiency may be healed with the intravenous administration of calcium and phosphorus even without repletion of vitamin D (55). It should be emphasized, however, that this finding does not exclude the possibility that vitamin D plays a direct role in the physiologic process of bone accretion. As discussed, vitamin D deficiency may result from poor intake, decreased intestinal absorption, or lack of exposure to ultraviolet light.

VDDR-I, also designated as pseudo-vitamin D–deficiency rickets, is inherited as an autosomal recessive disorder in which 25(OH)D-1α(OH)ase in proximal tubules is deficient due to mutation in its encoding gene. It is manifested by early hypocalcemia, hypophosphatemia, severe secondary hyperparathyroidism, and severe rickets. The serum concentrations of $1,25(OH)_2D_3$ are undetectable or very low, whereas 25(OH)D levels are normal or slightly elevated. The clinical abnormality can be reversed completely by the administration of pharmacologic doses of vitamin D or physiologic doses of $1,25(OH)_2D_3$. Linkage analysis in families with VDDR-I mapped the disease locus to chromosome 12q13-14. The synthesis of $1,25(OH)_2D_3$ from its precursor $25(OH)D_3$ is catalyzed by 25(OH)D-1α(OH)ase(1α[OH]ase), a mitochondrial P-450 enzyme in the proximal tubular cells. The cloning of 1α(OH)ase was achieved very late because of its very low gene expression and was reported only in 1997, many years after the cloning of 24(OH)ase that catalyzes by an alternative pathway for the metabolism of $25(OH)D_3$ to $24,25(OH)_2D_3$. 1α(OH)ase shares high homology with hepatic vitamin D hydroxylase. Because $1,25(OH)_2D_3$ may alter the hepatic metabolism of $25(OH)D_3$ and the $1,25(OH)_2D_3$-receptor (VDR) is present in the liver, the possibility that $1,25(OH)_2D_3$ acts by binding to its hepatic receptor to reduce the activity of hepatic vitamin D-25(OH)ase is plausible (45).

Table 6–5 Causes of Rickets and Osteomalacia

Group I: Low Serum Calcium–Phosphorus Product
Vitamin D deficiency
Vitamin D-dependent rickets, type I (1α-hydroxylase deficiency)
Vitamin D-dependent rickets, type II (vitamin D resistance)
Vitamin D-dependent rickets type III
Hypophosphatemic Rickets with Elevated FGF23

X-linked hypophosphatemic rickets
Tumor-induced osteomalacia
Autosomal dominant hypophosphatemic rickets
Autosomal recessive hypophosphatemic rickets
Osteoglophonic dysplasia
McCune–Albright syndrome (polyostotic fibrous dysplasia)

Hypophosphatemic Rickets due to Abnormal Tubular Transport

Hereditary hypophosphatemic rickets with hypercalciuria
Lowe oculocerebrorenal syndrome
X-linked recessive hypophosphatemic rickets (Dent disease)
Fanconi syndrome

Other Causes of Rickets and Osteomalacia

Excessive intake of phosphate-binding antacids
Hypophosphatemic nonrachitic bone disease
Renal tubular acidosis

Group II: Normal or High Serum Calcium–Phosphorus Product

Renal (uremic) osteomalacia
Hypophosphatasia
Bisphosphonates

The expression of renal 1α(OH)ase has been shown to be inhibited by its product 1,25$(OH)_2D_3$, and mice lacking 1,25$(OH)_2D_3$-receptor (VDR) develop abnormally high serum levels of 1,25$(OH)_2D_3$, suggesting that the expression of 1α(OH)ase is regulated by 1,25$(OH)_2D_3$ through liganded VDR.

The availability of cDNA and the gene structure that encodes the human 1α(OH)ase now makes it possible to analyze for inactivating mutations of 1α(OH)ase in patients with VDDR-I. Various mutations were identified in the 12q13-14 locus in patients with VDDR-I, suggesting that there is more than one founder for the 1α(OH)ase mutations in these patients (182).

Hypocalcemia and rickets refractory to 1,25$(OH)_2D_3$ were described as VDDR-II, also known as hereditary 1,25$(OH)_2D_3$-resistant rickets. This familial disorder is inherited by autosomal recessive transmission and is characterized by rickets, impaired intestinal absorption of calcium, hypocalcemia, and alopecia, which may reflect a defect in the physiologic

action of $1,25(OH)_2D_3$ in the skin. In contrast to VDDR-I, in type II, serum $1,25(OH)_2D_3$ is elevated, and the patients either respond to pharmacologic doses of $1,25(OH)_2D_3$ or do not respond at all. In some patients with this disorder, abnormal nuclear uptake, abnormal cytosol receptor binding of $1,25\text{-}(OH)_2D_3$, or both are present. These findings suggest that the mechanism of end-organ resistance is a defect in the receptor. However, this abnormality was not present in other patients, indicating a more distal postreceptor abnormality. In this regard, in one patient with type II vitamin D-dependent rickets with normal receptor function (receptor-positive resistance), failure of $1,25(OH)_2D_3$ to stimulate the enzyme $25(OH)_2D_3$-24(OH)ase was demonstrated (183). In normal people, $1,25(OH)_2D_3$ was shown to stimulate the formation of $24,25(OH)_2D_3$. The event may represent a step in the physiologic action of $1,25(OH)_2D_3$ that is lacking in some patients with type II vitamin D-dependent rickets (183–185). Mutations of VDR genes have been identified in some families with this abnormality. In one family, a nonsense mutation coding for a premature stop codon in exon 7 of the gene-encoding VDR was identified. In other families, the genetic abnormality consisted of a point mutation within the steroid binding domain of the VDR gene (186).

The skeletal lesions in VDR null mutant mice were largely reversed by normalizing ambient calcium and phosphate. This suggested that the skeletal abnormalities resulting from VDR ablation are caused by impaired intestinal absorption of calcium and phosphate. Thus, the absence of vitamin D action does not affect skeletal metabolism so long as mineral homeostasis is restored to normal.

A new type of vitamin resistance has been reported and termed “VDDR type III.” The phenotype is identical to VDDR-II, but the resistance to vitamin D results from overexpression of a heterogenous ribonucleoprotein that competes with normally functioning VDR–RXR dimer for binding to the vitamin D response element of the targeted gene (187,188).

Hypophosphatemia caused by excessive external losses of phosphorus may cause osteomalacia even in the presence of normal serum calcium concentration. Hereditary and acquired renal losses of phosphorus can be divided into three general groups: (1) hypophosphatemia caused by excessive phosphaturic action of phosphatonins, mainly resulting from circulating FGF23; (2) hypophosphatemia caused by a defect in NaPi

cotransporters; and (3) hypophosphatemia caused by enhanced proximal tubular response to PTH.

Phosphatonins include FGF23, secreted frizzled-related protein 4 (sFRP4), matrix extracellular phosphoglycoprotein (MEPE), and fibroblast growth factor 7 (FGF7).

The presence of a putative circulating phosphaturic substance other than PTH in patients with advanced CKD was foreseen in 1969. This prediction was based on studies in CKD patients who underwent total parathyroidectomy for secondary hyperparathyroidism. Fractional excretion of phosphorus that was very elevated before surgery failed to decrease after total removal of parathyroid glands. It was proposed that unidentified circulating factor(s) other than PTH that were present in these patients acted as inhibitors of tubular reabsorption of phosphorus in the absence of PTH (189). Twelve years later, in 1981, a patient was admitted to the neurology department with muscular paralysis and generalized bone pains and was found to have serum phosphate of 1.2 mg/dL with high urinary phosphorus, normal serum calcium, and an upper normal concentration of PTH. Bone biopsy showed osteomalacia. A mesenchymal giant cell tumor was resected from his left hip area. The patient recovered after surgery. An extract from the homogenate of the tumor that was infused into parathyroidectomized rats induced a profound phosphaturia. A similar infusion from an unrelated tumor that served as a control did not induce phosphaturia. The phosphaturic response was not associated with an increase in urinary cAMP, thus excluding PTH as the phosphaturic factor (190–194).

Phosphaturic humoral factor(s) causing tumor-induced osteomalacia (TIO), called phosphatonins, were extensively studied by the Mayo Clinic group (195,196). It was demonstrated that FGF23 is a causative factor of TIO. This factor was cloned from a tumor tissue following isolation of cDNA clones that were abundantly expressed only in the tumor. Administration of recombinant FGF23 decreased serum phosphate in mice within 12 hours. Chinese hamster ovary cells stably expressing FGF23 were subcutaneously implanted into nude mice, and hypophosphatemia with increased renal clearance of phosphate was observed.

Conditioned media from such tumors inhibited sodium-dependent phosphate transport in OK cells. Interestingly, the phenotype of TIO is shared by patients with inherited disorders of hypophosphatemic rickets, including autosomal dominant hypophosphatemic rickets (ADHR), X-linked hypophosphatemic rickets (XLH), and autosomal recessive hypophosphatemic rickets (ARHR). Sera of all the above patient groups

tested positive for phosphatonin activity (197). Further research work identified distinct members of the phosphatonin group from TIO patients, including FGF23, sFRP4, FGF7, and MEPE. FGF23 that was cloned in 2001 attracted most attention and was well characterized by lab animal experiments. Transgenic mice that overexpress FGF23 fully reproduce the abnormalities of patients with TIO and exhibit an important functional feature of FGF23 that is suppression of 25(OH)D-1α(OH)ase with consequent low levels of $1,25(OH)_2D_3$ and hyperparathyroidism. In this regard, it has been proposed that FGF-23 may serve as a counterregulatory hormone to $1,25(OH)_2D_3$ to maintain phosphate balance, in face of the effect of $1,25(OH)_2D_3$ to increase phosphate load by increasing intestinal absorption. FGF23 acts primarily as a phosphaturic hormone by inhibiting NaPi cotransporters, NaPiIIa, NaPiIIc, in the kidney, and possibly NaPiIIb in the small bowel. In mice, $1,25(OH)_2D_3$ increases FGF23 concentrations and its gene expression. Calcitonin suppresses FGF23 expression (42,197).

Deficiency of FGF23 causes renal phosphorus retention, hyperphosphatemia, increased $1,25(OH)_2D_3$ levels, soft tissue calcification, and defective bone mineralization. The latter has been attributed to the direct action of $1,25(OH)_2D_3$ on bone (197).

FGF23 binds to and activates FGF23 cognate receptors in cells that express Klotho. Klotho is a transmembrane protein that determines the specificity of tissue targeted by FGF23. In this regard, Klotho binds to FGF23 and converts the canonical FGF receptor to a specific receptor for FGF23. This enables high-affinity binding of FGF23 to all surface receptors in DCT where Klotho is expressed. FGF signaling is mediated by the mitogen-activated protein kinase (MAPK) cascade and phospho-ERK 1/2 (p-ERK 1/2). Klotho is produced in two isoforms by alternating splicing of the 5-exon gene. The first isoform is a single-pass transmembrane protein, with extracellular and cytoplasmic domains. Cleavage of the extracellular domain produces a cut segment found in circulation. The second isoform has only an extracellular domain without the transcellular portion and is secreted into circulation (197–199).

It is of interest that FGF23-Klotho-receptor signaling is localized in the DT, whereas its physiologic effect to reduce phosphorus reabsorption by decreasing NaPiII abundance is in the proximal tubule. The anatomical proximity between proximal and DT perhaps may enable a cross talk between both of them, thus generating a distal-to-proximal feedback. This perhaps can be mediated by a paracrine factor produced in the DT. TRPV5 activity is enhanced by Klotho in the DT independent of FGF23. Klotho

directly regulates PTH secretion in the parathyroid glands by recruiting Na^+/K^+ ATPase to the cell surface in response to low extracellular calcium concentration. It is likely that by reducing cytosolic sodium concentration by sodium pump, increased sodium gradient enhances NCX. Thus, calcium is pumped out of the cell in exchange for sodium that enters the cell down its gradient. In this regard, Klotho signaling may regulate PTH secretion by changing intracellular calcium (197).

Hereditary hypophosphatemic disorders may be divided into three groups—Group I: FGF23-dependent disorders; Group II: primary disorders of phosphorus transporters; and Group III: disorders due to hyperresponsiveness to PTH.

Group I: FGF23-Dependent Group of Hereditary Hypophosphatemic Rickets

X-Linked Hypophosphatemic Rickets

Hypophosphatemic vitamin D-resistant rickets is a sex-linked dominant disorder also known as XLH, in which renal tubular defects in phosphate reabsorption have been demonstrated (190,191). Serum $1,25(OH)_2D_3$ levels in patients with XLH are in the low-normal or slightly below normal range. Because hypophosphatemia is expected to stimulate α(OH)ase activity in the kidney, these relatively low values suggest that, in addition to a defect in tubular phosphate absorption, the response of 1α(OH)ase to low levels of serum phosphate also is impaired in XLH. This hypophosphatemic disorder is associated with mutation of the gene that encodes the phosphate-regulating endopeptidase homologue on the X chromosome (PHEX). PHEX is a type I cell surface zinc metallaprotease that is involved in the regulation of FGF23. PHEX cleaves small peptides such as ASARM (acidic serine- and aspartic acid-rich motif) peptide derived from MEPE, but the physiologically relevant peptide for PHEX that regulates FGF23 is unknown. Mutation in PHEX increases transcription of FGF23 in osteocytes. It is assumed that an unidentified substrate of PHEX accumulates and stimulates FGF23 gene promoter activity. The earlier suggestion that PHEX processes FGF23 was not borne out. Both the hypophosphatemia and the low $1,25(OH)_2D_3$ levels are related to high levels of circulating FGF23.

XLH occasionally responds to treatment with large doses of oral phosphate as well as to pharmacologic doses of vitamin D. The combined

administration of oral phosphate supplements with $1,25(OH)_2D_3$ is better than the administration of either alone for the cure of the bone disease in XLH. In hypophosphatemic mice (HYP mice), the animal model of the disease, the administration of phosphate cures the rickets but not the osteomalacia. The combined administration of phosphate supplements and $1,25(OH)_2D_3$ is necessary to achieve improvement of the osteomalacia as well (191,200). It is unknown whether a similar therapeutic response applies to the human disease.

The presence of a tumor may be responsible for excessive urinary loss of phosphate and hypophosphatemic rickets. The tumors that have been identified most frequently consist of mesenchymal and giant cells and often are present in bones. Excision of the tumor has been associated with reversal of the tubular leak of phosphate and cure of the bone disease in many patients (193). Recently, the beneficial effect of the administration of octreotide in a patient with TIO has been reported. It has been proposed that the syndrome is the result of a release of phosphaturic substance by the tumor. Indeed, tumor extracts elicited phosphaturic cAMP-independent effects when injected into animals (194,195). Likewise, in vitro, tumor extract inhibited NaPi coupled uptake using OK cells (196). FGF23 has been isolated from these tumors.

Hypophosphatemic nonrachitic bone disease is an entity that resembles X-linked hypophosphatemia, but it is clinically less severe with regard to bone disease, and there is no clear evidence of X-linked inheritance.

Autosomal Dominant Hypophosphatemic Rickets

ADHR is a phosphate-wasting disorder that maps to chromosome 12p13 and is characterized by short stature, bone pain, fracture, and lower extremity deformity. It is caused by mutations in the RXXR furin-like cleavage domain in FGF23 that makes it resistant to proteolytic inactivation by furin proprotein convertase. Thus, patients with ADHR missense mutation produce polypeptides that are less sensitive to protease cleavage than wild FGF23. ADHR mutations protect FGF23 from degradation, thereby elevating circulating concentrations of FGF23 and leading to phosphate wasting in ADHR patients.

Autosomal Recessive Hypophosphatemic Rickets

ARHR, a variant of hypophosphatemic rickets, is caused by an inactivation mutation of Dentin matrix protein 1 (DMP_1). DMP_1 is a

glycophosphoprotein that belongs to the small integrin-binding ligand N-linked glycoprotein family (SIBLING) and is expressed in bones and teeth, where it induces mineralization of extracellular matrix. Loss of DPM_1 results in increased transcription of FGF23 in osteocytes. DPM_1 null mice are a model of ARHR and have elevated circulating FGF23.

Osteoglophonic Dysplasia

Osteoglophonic dysplasia is an autosomal dominant bone dysplastic disorder caused by activating mutation of the FGR1 gene that may regulate FGF23 expression in bone and/or increase its phosphaturic effect in the kidney.

Hypophosphatemic Rickets with Hyperparathyroidism

This hypophosphatemic disorder features both rickets and hyperparathyroidism caused by parathyroid hyperplasia. The primary abnormality in this hypophosphatemic rickets is elevated levels of Klotho as a result of Klotho gene translocation. FGF23 levels are elevated in this disorder by a yet unknown mechanism. It has been proposed that the elevated levels play a role in parathyroid hyperplasia (198).

Additional Hypophosphatemic Disorders Associated with Elevated FGF23

Other hypophosphatemic disorders in which high levels of FGF23 have been reported to be elevated include McCune–Albright polyostotic fibrous dysplasia with activating mutation of $GNAS_1$. It is unclear whether the hypophosphatemia is caused by FGF23, which is elevated in 50% of patients, and/or by activation of the adenylate cyclase/cAMP pathway in the kidney. Epidermal nevus syndrome is caused by activating FGF23 mutations in the affected skin. Some patients with this syndrome have elevated FGF23 levels and hypophosphatemia.

Group II: Primary Disorders of Sodium-Phosphate Cotransporters

Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH)

HHRH is an autosomal recessive disorder characterized by

hypophosphatemia due to renal phosphorus wasting, increased serum levels of $1,25(OH)_2D_3$, increased intestinal absorption of calcium and hypercalciuria, rickets, and osteomalacia (201,202). It was originally assumed that this disorder is caused by a mutation in the NaPiIIa cotransporter gene, because the human phenotype is similar to that of mice with the deletion of NaPiIIa. Surprisingly, no mutation was identified in the NaPiIIa gene, but a mutation was found in the NaPiIIc cotransporter gene. Thus, HHRH is caused by the single-nucleotide deletion in the NaPiIIc cotransporter gene (203).

Group III: Hypophosphatemic Disorders Secondary to Hyperresponsiveness to PTH

NHERF1 Mutations and Renal Responsiveness to PTH

NHERF1 is a scaffold protein that links with NaPi cotransporters by interacting with the *C*-terminal tail of NaPiIIc and NaPiIIa and anchors them within apical cell membranes. NHERF1 phosphorylation by PTH leads to internalization of Na/Pi cotransporters followed by their degradation in lysosomes. NHERF1 in cultured cells attenuates PTH-induced formation of cAMP. Thus, NHERF1 plays an important role in tubular phosphorus transport. Inactivating missense mutations in NHERF1 have been identified in patients with hypercalciuria and nephrolithiasis that exhibited reduced reabsorption of phosphorus below lower limits of normal as well as causing hypophosphatemia. In vitro experiments showed that these mutations potentiated PTH-induced cAMP generation and consequently caused inhibition of phosphorus transport. Patients with this abnormality presented with reduced bone mineralization and elevated $1,25(OH)_2D_3$. Thus, inactivating mutations in NHERF1 area are a new class of pathogenic factors in hypophosphatemia (204).

Dent Disease or X-Linked Hypercalciuric Nephrolithiasis

Dent disease is characterized by low-molecular-weight proteinuria, hyperphosphaturia, and hypercalciuria, which eventually lead to kidney stones, nephrocalcinosis, and renal failure. This hereditary proximal tubulopathy is caused by mutation of the chloride channel 5 (CLCN5) gene that encodes the voltage-gated chloride channel and chloride proton antiporter. This mutation results in a defect in proximal tubular endocytosis. This leads to reduced PTH endocytosis with increased tubular

PTH concentration available to bind to its PTH1R receptors. This in turn potentiates the tubular effect of PTH, causing increased endocytosis of NaPiII cotransporters with reduced phosphorus reabsorption, hyperphosphaturia, and hypophosphatemia (205).

Low oculocerebrorenal syndrome (OCRL1) is caused by mutations of genes that encode phosphatidylinositol 4,5-bisphosphonate 5-phosphatase, which also produces a defect in endocytosis. OCRL1 patients feature more severe hypophosphatemia, bone disease, and tubular proteinuria than those in patients with Dent disease.

Jansen Metaphyseal Chondrodysplasia

Jansen chondrodystrophy is characterized by dwarfism with short limbs, bowing of long bones, mild hypercalcemia, nephrolithiasis, hypophosphatemia, and low serum PTH level, but elevated urinary cAMP excretion. It is caused by activating, gain-of-function mutations of PTH/PTHrP receptor and is inherited as an autosomal dominant trait. It is associated with increased proliferation and delayed maturation of chondrocytes.

OTHER METABOLIC ABNORMALITIES ASSOCIATED WITH RICKETS AND OSTEOMALACIA

Even though calcium deficiency per se has not been recognized as a cause of osteomalacia in humans, several reports suggest that this abnormality may cause rickets in babies and children (206). It is possible that in these circumstances calcium deficiency–induced secondary hyperparathyroidism may lead to vitamin D deficiency. High circulating PTH levels stimulate renal α(OH)ase, resulting in high $25(OH)_2D_3$ levels. Thus, the proposed mechanism of this abnormality is increased breakdown of $25(OH)_2D_3$ by high levels of $1,25(OH)_2D_3$, causing a state of vitamin D deficiency (91).

Fanconi syndrome is associated with multiple defects in tubular transport. The phosphaturia and renal tubular acidosis associated with this syndrome may be primarily responsible for the occurrence of the hypophosphatemic rickets (207). The exact cause of osteomalacia in patients with renal tubular acidosis is not entirely clear. Another possible mechanism of osteomalacia in Fanconi syndrome is vitamin D deficiency caused by failure to absorb vitamin D bound to a protein carrier in the proximal tubule. Acidosis per se leads to the development of osteoporosis rather than osteomalacia in animals. It has been proposed that

hypercalciuria associated with renal tubular acidosis lowers serum calcium and stimulates the secretion of PTH, which in turn causes excessive urinary losses of phosphorus, with hypophosphatemia and osteomalacia. An alternative explanation for the phosphaturia is that acidosis directly increases urinary excretion of phosphorus. Osteomalacia also has been reported in patients with systemic acidosis following ureterosignoidostomy some years earlier (208). The acidosis occurs as a result of fecal bicarbonate losses in this latter situation. The fact that osteomalacia associated with renal tubular acidosis may be cured in some patients with the correction of the acidosis emphasizes the potential role of acidosis in this disorder. Both osteoblasts and osteoclasts sense extracellular H^+ level, by a G protein–coupled receptor OGR1. Stimulation of OGR1 causes enhanced bone resorption; however, augmented bone formation that exceeds resorption leads to a net increase in bone mass. This experimental paradox requires further research (209). Osteomalacia also has been reported in patients with phosphate depletion caused by excessive intake of phosphate-binding antacids and excessive use of laxatives.

All previously discussed disorders causing osteomalacia share one common feature, namely, a reduced serum calcium–phosphorus product that may be responsible for the failure of bone matrix to mineralize. However, osteomalacia in chronic renal failure develops in the presence of a high serum calcium–phosphorus product. In these patients, the mineralization defect may be related to intrinsic abnormalities of the organic matrix, a circulating inhibitor of mineralization, or deficiency of a specific metabolite of vitamin D. Aluminum toxicity causes a mineralization defect in some patients with hemodialysis-associated osteomalacia (210). In osteomalacia associated with hypophosphatasia, the high concentration of pyrophosphates may block bone mineralization despite the presence of normal or high concentrations of serum calcium and phosphorus. Osteomalacia may also develop in association with the administration of bisphosphonates, which share common chemical properties with pyrophosphates.

Osteomalacia may evolve in patients receiving long-term total parenteral nutrition. A normal or slightly elevated serum concentration of calcium and phosphate has been reported in this disorder. In one study, osteomalacia developed during supplementation with vitamin D. Associated abnormalities include hypercalciuria, exceeding the amount of calcium intake, mildly elevated concentrations of serum calcium and serum $25(OH)D_3$, and low serum $1,25(OH)_2D_3$ and serum PTH concentrations. Elimination of vitamin D supplements from the formula of

parenteral nutrition reversed the biochemical and hormonal abnormalities and led to recovery from the bone disease (211). This sequence suggests that either vitamin D toxicity or hypersensitivity to vitamin D with consequent loss of mineral in the urine was responsible for osteomalacia. The observed suppression of PTH secretion could play a role in the reduced bone turnover and increased losses of calcium in the urine, which could not be replaced because of the absence of the intestinal supply of calcium. Other studies proposed that either low $1,25(OH)_2D_3$ or aluminum toxicity could be causally related to the observed osteomalacia (212,213). Thus, the total parenteral nutrition–induced osteomalacia remains a poorly understood entity.

OSTEOPOROSIS

Normal bone remodeling is based on matching of bone resorption with bone formation. Each year, 25% of trabecular bone is resorbed and replaced in adults. The turnover rate of cortical bone is substantially slower. Under normal conditions, the bone renewal process proceeds in cycles of resorption followed by formation. During new bone formation, the osteoblasts lay down type I collagen in longitudinal layers. The collagen molecules are then interconnected by pyridinoline cross-linking to provide strength. Two stages of mineralization then follow. First, hydroxyapatite crystals are deposited between the collagen fibrils. The second stage proceeds over several months as more mineral is added to the bone. The constant bone turnover helps repair microfractures and remodels the bone in response to stress. The quantitative coupling between resorption and formation helps maintain normal bone mass. The hallmark of osteoporosis is loss of bone mass caused by imbalance between bone resorption and formation. Loss of gonadal function and aging are the two most important conditions leading to osteoporosis. The former is known as postmenopausal osteoporosis, and the latter as senile osteoporosis.

Peak Bone Mass

Osteoporosis is characterized by low bone mass and disrupted bone architecture, which leads to reduced bone strength and increases the risk of fractures (Table 6-6). Therefore, prevention of low bone mass is of prime importance. One of the means of reaching this goal is to increase the peak bone mass buildup during adolescence. Adolescence is a crucial time for the development of bone mass. Bone mass increases with age throughout

childhood, and it peaks by late adolescence and early adulthood. Bone mass accretion during puberty appears to be critical in the development of peak bone mass. Peak bone mass is regarded as a major determinant of osteoporosis in later life (214).

The bone mass remains stable after attainment of peak bone mass under normal conditions. An exception to this rule is the appearance of pregnancy-associated osteoporosis. It consists of four variants: (a) idiopathic osteoporosis of pregnancy, (b) transient osteoporosis of the hip in pregnancy, (c) postpregnancy spinal osteoporosis, and (d) lactation-associated osteoporosis. Whether these are truly independent conditions or they relate specifically to pregnancy remains to be determined. The most important feature of all types of pregnancy-associated osteoporosis is complete recovery without residual damage. Heparin-induced osteoporosis in pregnancy also is reversible after discontinuation of heparin (215).

An annual loss of 0.3% to 0.5% of bone mass may occur starting during the fourth to fifth decades of life. After menopause, the rate of bone loss may increase 10-fold. Loss of bone mass following menopause is characterized by increased bone turnover, featuring both increased bone resorption and increased bone formation. The osteoclastic resorbing activity, however, exceeds the osteoblastic bone-forming activity, resulting in net loss of bone mass. By contrast, the osteoporosis associated with aging, senile osteoporosis, is characterized by low bone turnover (216). The major feature of aging osteoporosis is reduced osteoblastic activity with reduced supply of osteoblasts. Thus, the amount of bone formed during each remodeling cycle is reduced, leading to a net decrease in bone mass. Additional differences between postmenopausal and senile osteoporosis are that the trabecular bone is affected mainly in the former, whereas cortical and trabecular bones are affected equally in the latter. Estrogen deficiency is the underlying mechanism of postmenopausal osteoporosis. The deficiency of estrogen creates an imbalance in bone metabolism with at least two known abnormalities. First, the resorptive effect of PTH is augmented in the absence of estrogen, with no change in bone formation. Second, estrogen suppresses the production of IL-6 by osteoblastic cells. IL-6 is an osteoclast-activating cytokine (217). Thus, excessive formation of IL-6 leads to excessive bone loss in postmenopausal osteoporosis (218). Serum levels of PTH and calcitriol are low in patients with postmenopausal osteoporosis. PTH levels are increased and calcitriol levels reduced in senile osteoporosis. There is reduced intestinal calcium absorption in both conditions. Estrogen therapy in postmenopausal osteoporosis leads to an increase in plasma calcitriol

levels and improves intestinal absorption of calcium (216,217).

Table 6–6 Clinical Forms of Osteoporosis

Generalized, primary	Generalized, secondary
Type I: Postmenopausal	Corticosteroid
Type II: Senile	Cushing syndrome
Type III: Idiopathic	Hyperthyroidism
Juvenile	Rheumatoid arthritis
Adult	Long-term heparin administration
In pregnancy	Alcoholism
Local	Anorexia nervosa
Transitory migrant osteoporosis	Hypogonadism
Fracture and immobilization	Malabsorption
Neurogenic immobilization	Acidosis
Transient osteoporosis of the hip in pregnancy	Cirrhosis of liver
	Vitamin C deficiency
	Lactation-associated osteoporosis
	Space travel

Low bone turnover, which is characteristic of senile osteoporosis, also is present in other types of secondary osteoporosis, including steroid-induced osteoporosis, alcohol-induced osteoporosis, osteoporosis associated with malabsorption and chronic liver disease, osteoporosis associated with anorexia nervosa, immobilization-induced osteoporosis, and idiopathic juvenile osteoporosis with and without hypercalciuria. On the other hand, osteoporosis associated with premature menopause, anovulatory cycles, primary hyperparathyroidism, secondary amenorrhea, and male hypogonadism is associated usually with high bone turnover. It is interesting that the two variants of osteoporosis differ also in the abnormalities in microarchitecture. In the high turnover type, the changes consist of thinning and loss of trabecular elements, reduced connectedness, erosions, and penetration of the trabeculas with total disruption of the architecture; in the low bone turnover type, the only change is thinning of the trabecules with loss of horizontal trabecules.

Bone mass is strongly correlated with compressive strength. However, there is a considerable overlap in bone density values between subjects with and without fractures. This emphasizes the importance of factors other than bone mass in the pathogenesis of fractures. These include bone

microarchitecture, composition of bone matrix, composition of bone mineral, and factors such as trauma. In this regard, it is of interest that patients with elevated serum levels of homocysteine are at very high risk of osteoporotic fractures. In vivo and in vitro studies suggest that homocysteine interferes with collagen cross-linking in bone, leading to abnormal bone matrix (219).

Even though the vast majority of osteoporotic fractures occur in patients with postmenopausal osteoporosis and elderly persons, it is noteworthy that the association of low bone mass with the occurrence of fractures has been recorded in younger people. It has been shown that athletes with stress fracture had lower bone mineral than did well-matched control athletes. Likewise, there was a good correlation between menstrual irregularity, reduced BMD, and stress fractures. A positive correlation between calcium intake and BMD was demonstrated in young individuals.

Bone Densitometry

Bone densitometry represents a major advance in the management of osteoporosis. The introduction of advanced technology to assess the bone density has provided clinicians with a valuable tool to assess patients at risk of fractures and monitor response to therapy. These methods include the use of dual energy X-ray absorptiometry (DXA), ultrasonic measurements (SOS), computerized X-ray tomography, and other methods. The indications for BMD measurements recommended by the Scientific Advisory Board of the National Osteoporosis Foundation in the United States are: (a) estrogen deficiency, (b) vertebral deformity and radiographic osteopenia, (c) asymptomatic primary hyperparathyroidism (reduced bone density is an indication for parathyroid surgery), and (d) monitoring of therapy. Optional indications include the presence of several minor risk factors such as genetic factors, alcohol intake, high caffeine intake, smoking, and reduced physical activity.

It is interesting that in recent genetic studies, polymorphism of the VDR gene has been linked with BMD in twin studies. It has been shown in postmenopausal women that allelic variants in the gene encoding the VDR can be used to predict differences in bone density. The molecular mechanism by which bone density is regulated by the VDR is not certain, although allelic differences in the three untranslated regions may alter mRNA levels. It has been proposed that the use of this genetic marker could allow earlier intervention in those with increased risk of osteoporosis (218). More recent reports have emphasized that the VDR gene acts

predominantly to determine peak bone mass and that other genes are likely to be involved in the regulation of bone loss after menopause (220).

TREATMENT OF OSTEOPOROSIS

Undoubtedly, measures aimed at prevention of osteoporosis are most valuable, because in many cases the disease is irreversible and refractory to therapy. Achievement of adequate peak bone mass may be facilitated with adequate intake of calcium and vitamin D, good physical activity, and early detection and treatment of predisposing diseases. Nonsmoking and moderation in alcohol and caffeine intake is recommended.

The therapeutic goals are dual in established osteoporosis: to increase bone formation and decrease bone resorption in order to maintain adequate bone mass and prevent osteoporotic fractures. There is a positive correlation between physical activity and bone density; therefore, gravity exercises and muscle-strengthening exercises should be encouraged.

Estrogen inhibits bone resorption, prevents bone loss, and may even increase bone mass in postmenopausal women. The daily dose of conjugated estrogen is 0.625 mg; it may be given in conjunction with progesterone with good effect. Administration of estrogen prevents not only bone loss but also vertebral and femoral fractures. It is recommended to treat with estrogen for at least 5 years. Reports on the incidence of breast cancer in estrogen-treated women need to be taken into consideration (221).

Bisphosphonates and calcitonin reduce bone turnover and, like estrogens, may potentially lead to increases in bone mass as a result of filling the remodeling space. Estrogens affect cortical and cancellous bone equally, whereas calcitonin and bisphosphonates mainly affect cancellous bone (221–224).

The bisphosphonate, alendronate (Fosamax), gained popularity in the treatment of postmenopausal osteoporosis. Our clinical follow-up experience based on a large population of postmenopausal women shows favorable therapeutic response in 60% to 65% of patients. The drug was discontinued in about 35% to 40% either because of lack of efficacy or because of intolerance to adverse side effects mainly related to the upper GI tract. Interestingly, alendronate failed to benefit patients with hypothyroidism receiving hormonal thyroid replacement.

Long-term follow-up studies showed that the therapeutic effects of alendronate on bone density and on bone fractures were sustained over 10 years (225). Concern about the quality of the bone exposed to

bisphosphonates has been expressed by some investigators. This concern addressed the fact that bisphosphonates are not “bone builders” but “bone hardeners.” Bone biopsies from patients treated with bisphosphonates show that the mineralizing surface that reflects bone formation is markedly reduced and that the bone volume does not change significantly. The mineral inside the bone is more densely packed; therefore, the bone density on DXA is increased (226). A recent study exploring bone biopsies in patients treated for 3 years with alendronate revealed a peculiar histology showing increased number of giant hypernucleated osteoclasts undergoing protracted apoptosis. This finding is poorly understood (227).

Fluoride increases cancellous bone density. The quality of the bone may be abnormal with fluoride, however, resulting in reduced strength despite increased mass (224,228). Oral calcium supplements, with and without vitamin D (including oral calcitriol), have been reported to be beneficial in certain studies. Obviously, the presence of vitamin D deficiency, which is common in the elderly, needs to be corrected with vitamin D supplements.

All therapeutic interventions that address the foregoing are based on the antiresorptive effects of therapeutic agents. By contrast, intermittent recombinant PTH (hPTH-[1-34]) administration has an anabolic effect on bone metabolism because it stimulates bone formation. Older therapeutic trials with alternating calcium and phosphorus infusions that achieved increases in bone volume in fact induced cyclical variations in serum PTH concentrations, similar to intermittent PTH injections (229).

Once-daily injections of hPTH-(1-34) increase the expression of the master osteogenic transcription factor Runx2, which increases osteoblast numbers and thereby enhances bone formation. Intermittent hPTH-(1-34) also increases the decoy protein osteoprotegerin (OPG) expression and reduces osteoclast activity. On the other hand, continuous infusion of PTH as in primary hyperparathyroidism has the opposite effect. It increases the RANK ligand and reduces OPG, resulting in increased activity of osteoclasts with increased bone resorption and an increase in serum calcium level (230).

A controlled, randomized clinical trial with 1,637 postmenopausal women with osteoporosis demonstrated that once-daily injection of PTH over 21 months increased bone formation and bone mass and decreased the risk of fractures. Sclerostin, a negative regulator of bone formation, is synthesized and secreted by osteocytes. Sclerostin acts by suppressing the Wnt/catenin signaling pathway that leads to inhibition of bone formation. Romosozumab, a monoclonal antibody that binds sclerostin, increases

bone formation and decreases bone resorption. In postmenopausal women with osteoporosis, Romosozumab increased bone density and reduced the risk of vertebral fractures (231).

CHRONIC RENAL DISEASE

Hyperplasia of the parathyroid glands was reported in the early part of this century in autopsies of patients dying of uremia. In 1943, a form of rickets that failed to respond to physiologic doses of vitamin D but responded to pharmacologic doses was reported in children with renal insufficiency (231). These preliminary observations stimulated the evaluation of two major skeletal abnormalities associated with chronic renal disease, namely, osteitis fibrosa cystica and osteomalacia.

Both biochemical studies and measurements of circulating immunoreactive PTH suggest that hyperactivity of parathyroid glands is present in the early stage of chronic renal disease. Assuming that a decrease in serum calcium concentration is the stimulus for secondary hyperparathyroidism in chronic renal failure, several factors may cause the hypocalcemia. Loss of functioning nephrons with a decreased filtered load of phosphorus leads to retention of phosphorus. The resulting increase in serum phosphorus concentration, with a reciprocal decrease in serum calcium concentration, stimulates the secretion of PTH. The increase in parathyroid activity corrects the hyperphosphatemia by decreasing tubular phosphate reabsorption and increasing urinary excretion of phosphorus and returning both serum phosphorus and calcium toward normal, but at the expense (“trade-off”) of increasingly rising serum levels of PTH (232). The hypothesis of the pathogenesis of secondary hyperparathyroidism in chronic renal failure is supported by studies in chronically azotemic dogs in which phosphate restriction prevented the development of secondary hyperparathyroidism. However, the major assumptions on which the “trade-off” hypothesis was based were not fulfilled entirely. First, no evidence was presented showing a rise in serum phosphorus in patients with early renal failure. In fact, these patients exhibit a normal or even low serum phosphate, with normal serum calcium. Likewise, sequential sampling of serum phosphate failed to demonstrate transient rises in serum phosphorus or decrements in serum calcium. Second, the patients with early renal failure do not show phosphate retention but rather have an increased ability to excrete phosphorus loads. The fact that phosphate restriction was shown to reverse the rise in PTH cannot be used as favoring the trade-off hypothesis. Phosphate levels per se, independent of

serum calcium, have been shown to alter PTH secretion (71,72).

Reduced intestinal absorption of calcium owing to acquired resistance to vitamin D has been proposed as a fundamental abnormality in chronic renal failure (233). This possibility was supported by studies indicating that the conversion of $25(OH)_2D_3$ to $1,25(OH)_2D_3$ takes place in the kidney and that serum levels of $1,25(OH)_2D_3$ are reduced in patients with chronic renal failure. It has been reported that physiologic doses of $1,25(OH)_2D_3$ improve the abnormal GI absorption of calcium in patients with chronic renal failure. These findings, however, apply to advanced renal disease. Intestinal absorption of calcium is normal in early renal failure, whereas serum PTH is already elevated.

Evidence has been advanced demonstrating a direct inhibitory effect of physiologic amounts of $1,25(OH)_2D_3$ on the synthesis of PTH in vivo at the genomic transcriptional level. Thus, reduced $1,25(OH)_2D_3$ levels in chronic renal failure could be responsible for the increased synthesis and secretion of PTH (70). It is noteworthy, however, that in early renal failure serum levels of $1,25(OH)_2D_3$ are variable, normal, low, or even elevated. It has been claimed that the presumably "normal" $1,25(OH)_2D_3$ levels measured in early renal failure may in fact be abnormally low relative to the elevated PTH level. In this regard, it has been demonstrated that the administration of calcitriol or dietary phosphate restriction in early renal disease results in normalization of serum PTH level. These observations do not necessarily prove conclusively that calcitriol deficiency is the mechanism of secondary hyperparathyroidism.

An additional proposed mechanism of the evolution of secondary hyperparathyroidism is altered number or binding affinity of VDRs for $1,25(OH)_2D_3$ resulting in a blunted response of parathyroid glands to the inhibitory effect of $1,25(OH)_2D_3$ and uninhibited synthesis of PTH. Although reduced density and number of VDRs have been demonstrated in hyperplastic glands removed from uremic patients, this has not yet been demonstrated in patients with early renal failure (234).

Parathyroid glands from uremic patients require higher ambient calcium concentrations than normal glands to suppress the secretion of PTH. Thus, the set point for calcium, that is, the concentration of calcium required to inhibit 50% of maximal PTH secretion, is shifted to the right. This abnormality of response to calcium can be corrected partially by treatment with calcitriol. The possibility that defects in the CaSR might be the mechanism of secondary hyperparathyroidism in early renal failure

requires further evaluation. It is of interest that vitamin D deficiency, as defined by low levels of $25(OH)D_3$, is more common than assumed in the past. Sustained repletion of vitamin D may reverse secondary hyperparathyroidism. It has been proposed that 24(OH)D is indicated before administration of vitamin analogs.

The calcemic response to PTH in chronic renal failure is blunted. This could represent downregulation of PTH receptors in the bone. It has been shown that this abnormality can be reversed after parathyroidectomy, suggesting that high PTH levels may play a role in the blunted calcemic response. The skeletal resistance to PTH has been demonstrated both in early and advanced renal insufficiency (232).

FGF23 is markedly increased in CKD (235). FGF23 levels show an early rise in CKD, perhaps even before the rise in PTH. The rise in FGF23 in early renal failure may explain hypophosphatemia that has been observed in some patients at an early stage of CKD. FGF23 may play an important role in the high fractional excretion of phosphorus that is observed in all stages of CKD and that remains elevated after total parathyroidectomy (189). Likewise, the reduced levels of $1,25(OH)_2D_3$ in advanced CKD could be partly caused by the inhibitory effect of FGF23 on 25(OH)D-1α(OH)ase in the kidney and thus contribute to secondary hyperparathyroidism. It has been proposed that FGF23 may also exert a direct effect on bone mineralization independent of serum phosphate. A recent epidemiologic study has shown that increased FGF23 levels appear to be independently associated with mortality among patients who are beginning hemodialysis treatment. A recent animal study suggests that FGF23 directly inhibits the osteoblasts in the Wnt pathway contributing to bone loss in CKD. This effect of FGF23 seems to be mediated by Klotho/MAPK-driven processes involving DKK1 induction (236).

As renal failure advances, hyperphosphatemia develops and assumes a major role in the aggravation of secondary hyperparathyroidism. Likewise, the serum levels of $1,25(OH)_2D_3$ decrease, and the intestinal absorption of calcium is low. In many patients with advanced renal failure, the hyperplastic parathyroid glands do not respond to physiologic regulation and become refractory to treatment. This sets the stage for the emergence of “tertiary” or “autonomous” hyperparathyroidism, which may require surgical removal of excessive parathyroid tissue. In these circumstances, hypercalcemia may develop as a result of loss of feedback regulation. The combined elevation of serum calcium and phosphorus levels with an increase in their product may lead to metastatic calcifications.

Recent studies have examined the clonality of hyperplastic parathyroid glands from patients with autonomous secondary uremic hyperparathyroidism. Tumor monoclonality was demonstrated in 64% of patients with uremic refractory hyperparathyroidism. Monoclonality implied that somatic mutation of certain genes controlling cell proliferation occurred in a single parathyroid cell, conferring a selective growth advantage to the transformed cell, leading to neoplastic transformation (237).

RENAL BONE DISEASE

The recent conferences of Kidney Disease: Improving Global Outcomes (KDIGO)'s Global Mineral and Bone Initiative recommended that (1) the term "renal osteodystrophy" be used exclusively to refer to alterations in bone morphology that are associated with CKD and (2) the term "chronic kidney disease–mineral and bone disorder" (CKD–MBD) be used to describe the broader clinical syndrome that develops as a systemic disorder of mineral and bone disorder as a result of CKD. The following discussion will focus on some features of renal osteodystrophy.

Secondary hyperparathyroidism causes the development of osteitis fibrosa cystica, which presents radiographically as subperiosteal bone resorption. These lesions are most commonly seen in the middle phalanges of the hands, distal ends of the clavicles, and proximal ends of the tibia. Cystic lesions and brown tumors may be a radiographic feature of hyperparathyroid bone disease.

Osteitis fibrosa cystica is the most common skeletal abnormality in both adults and children with chronic renal failure. This hyperparathyroid osteodystrophy is characterized by rapid bone turnover, featuring both increased osteoclastic resorption and increased osteoblastic bone formation. The rapid bone turnover can be demonstrated by an increased number of double-tetracycline labels. The rapid bone turnover is also associated with marrow fibrosis and an increased amount of woven osteoid. It differs from normal lamellar osteoid in that there is a haphazard arrangement of collagen fibers. Although woven osteoid can be mineralized, the calcium is deposited as amorphous calcium and phosphate instead of hydroxyapatite. The presence of woven bone is characteristic of states of active bone and is visualized with a polarizing microscope. Advanced forms of osteitis fibrosis cystica present an abnormal bone architecture in which vast quantities of normal mineralized bone are replaced with fibrous tissue, with multiple cysts, and with woven bone that

is mechanically defective. This leads to serious skeletal deformities and fractures.

Another interesting radiographic feature of renal osteodystrophy is osteosclerosis. This entity is associated with increased density of bone, as assessed by X-ray examination, and is most frequently observed in the vertebrae.

In many instances, the secondary hyperparathyroidism seen in advanced renal insufficiency may be reversed with sustained control of serum calcium and phosphorus, which may be accomplished by the use of vitamin D, phosphate-binding antacids, and the administration of calcium carbonate, sevelamer, lanthanum, and others. It has been shown that with continuous control of calcium and phosphorus levels in the serum, the level of circulating PTH decreases and radiographically observed bone lesion may resolve (Fig. 6-9) (238). Metastatic calcifications resolve with this regimen as well (239). This therapeutic approach may not be successful in some patients because of extremely severe hyperplasia of the parathyroid glands, and subtotal parathyroidectomy is then the treatment of choice.

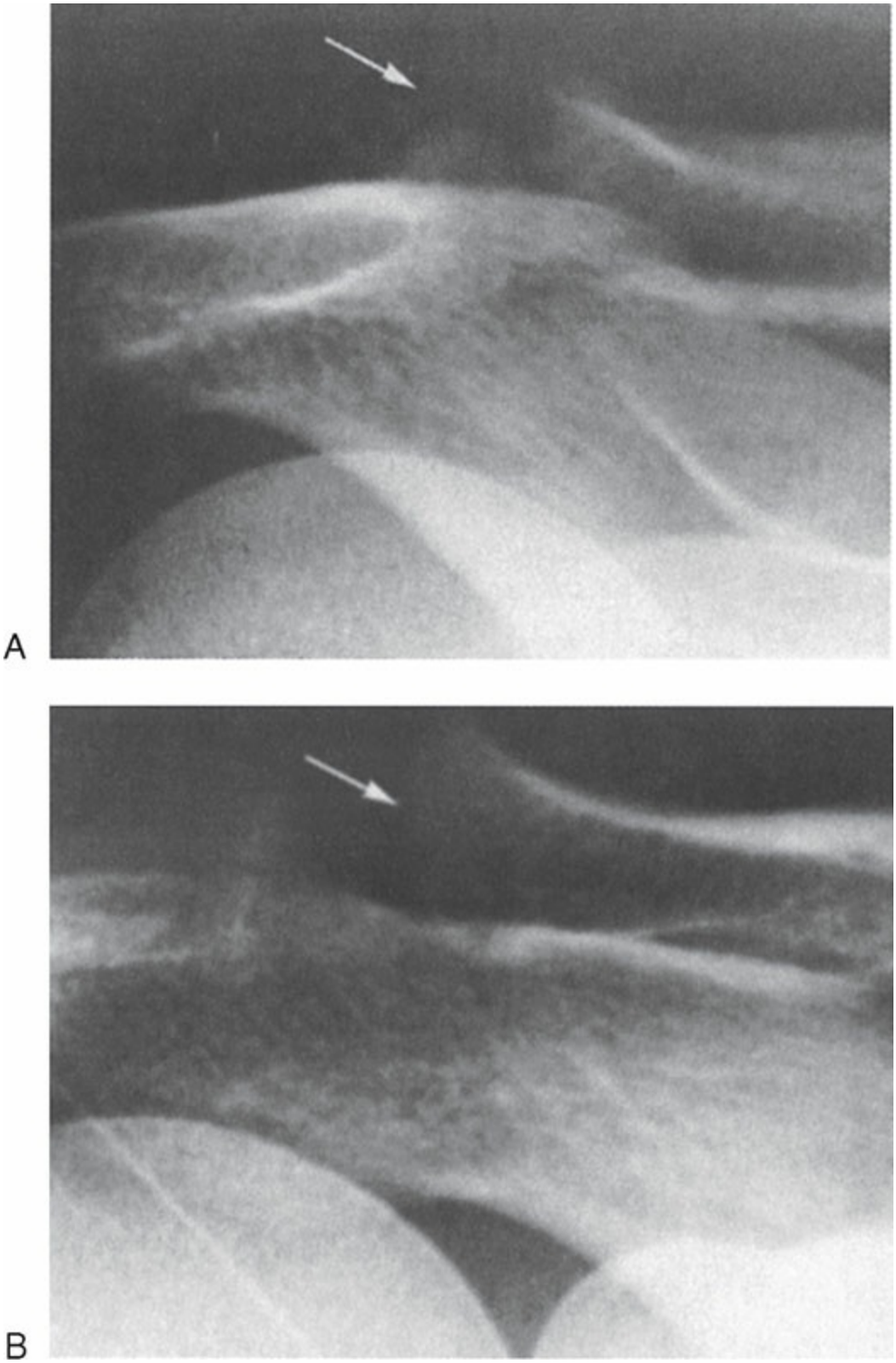


Figure 6–9 (**A**) Subperiosteal resorption at the lateral end of the clavicle (*arrow*), with an irregular appearance and loss of cortical outline. (**B**) Healing of bone, with filling of the defect and reappearance of cortical outline after therapy with calcium carbonate and aluminum hydroxide. (*Arrow* indicates distal clavicle.)

Intravenous use of pharmacologic doses of calcitriol, in intermittent

doses, has been recommended as a highly efficient way to achieve better suppression of parathyroid hyperactivity in patients with chronic renal failure (240). Indeed, many studies have confirmed the therapeutic efficacy of the intravenous intermittent administration of calcitriol in suppressing PTH levels. The effect of this form of therapy on bone histomorphometry is not entirely clear. In a limited number of studies, intravenous therapy with calcitriol led to marked suppression of bone turnover, with marked reduction in bone formation but with variable effect on osteoclastic resorption (241). It is noteworthy that the initial clinical studies comparing the efficacy of the intermittent intravenous administration of calcitriol with the oral route of administration were uncontrolled. The results of a recent controlled randomized study comparing long-term oral administration of calcitriol with intermittent intravenous administration showed that they were equivalent in the treatment of secondary hyperparathyroidism. Additional observations of that study were that treatment of severe secondary hyperparathyroidism remains difficult regardless of the route of administration. Moreover, the dose of calcitriol was limited by side effects (242).

Additional intravenous preparations of active vitamin D analogs including paracalcitol (Zemplar), and doxercalciferol (Hectorol) are widely used in the treatment of secondary hyperparathyroidism in patients undergoing dialysis. These analogs may have an advantage over older intravenous forms of vitamin D by primarily targeting parathyroid glands and exerting a minimal effect on intestinal phosphorus absorption (243).

Calcimimetics are allosteric activators of CaSR. Their binding to CaSR stimulates signaling to mobilize and increase intracellular calcium and decrease parathyroid secretion and synthesis. As opposed to vitamin D analogs, calcimimetics do not directly alter intestinal absorption of calcium and phosphate, and can lower PTH without increasing circulating phosphorus and calcium. Cinacalcet is the only available calcimimetic drug. Bone distomorphometric studies in dialysis patients showed that treatment with cinacalcet lowered PTH, improved bone histology, and reduced bone turnover in most of the studied patients with secondary hyperparathyroidism (244).

A previous trial demonstrated a beneficial effect of oral administration of $24,25(OH)_2D_3$ when combined with 1α(OH)D on hyperparathyroid bone disease in patients on chronic hemodialysis (245). The potential therapeutic effort of this regimen is uncertain and requires further investigation.

Two additional distinct forms of bone disease in patients with chronic

renal disease are osteomalacia and adynamic or aplastic bone disease. A so-called vitamin D-refractory rickets was described in the 1940s in children with advanced renal insufficiency. This form of mineralization defect most likely resulted from deficiency of calcitriol. Osteomalacia may be present in predialysis patients with chronic renal failure also. Most of these patients present with hypocalcemia and normophosphatemia; their kidney disease usually is interstitial and/or obstructive uropathy. Many respond favorably to calcitriol administration.

Aluminum has been recognized as a toxic factor involved in the pathogenesis of uremic osteomalacia. The aluminum-related lesion is characterized by the presence of excessive amounts of inactive osteoid and very low bone turnover. The staining of bone for aluminum is usually strongly positive in states of aluminum overload. Although this type of bone disorder has been described mainly in patients undergoing chronic dialysis, it may occur in uremic predialysis patients. Aluminum-associated osteomalacia is a very symptomatic bone disease manifested by severe skeletal pains and fracture (246). Aluminum can be mobilized and removed with the use of the chelating agent deferoxamine mesylate, which bind aluminum. Removal of aluminum leads to recovery. Aluminum-induced bone disease has become less frequent with the restricted use of aluminum-based phosphate binders and the widespread employment of water treatment with reverse osmosis in dialysis units.

Adynamic bone at present is one of the enigmas in the spectrum of renal bone disease. It is characterized by very low bone turnover (low or absent tetracycline uptake) but with no obvious abnormalities in the static parameters of bone histomorphometry. This form of bone pathology has been variously attributed to suppression of parathyroid function by high calcium concentration in dialysate solution or by excessively high levels of circulating calcitriol such as those that follow the intravenous administration of pharmacologic doses of this metabolite of vitamin D (247). It is interesting that patients with the aplastic bone lesion, similar to those with osteomalacia, frequently develop hypercalcemia following oral or intravenous calcium loads. Thus, both aluminum-associated osteomalacia and adynamic bone are characterized by poor buffering capacity to exogenous calcium. Recent studies indicate that aplastic bone lesion is the prevalent bone abnormality in patients treated with chronic ambulatory peritoneal dialysis and diabetic end-stage renal disease (248). PTH levels are relatively low in both groups. Likewise, low turnover bone lesion has been recently recognized as a common abnormality in patients undergoing chronic hemodialysis.

There are mixed types of renal osteodystrophy, which combine elements characteristic of more than one defined lesion in addition to the discrete forms of bone disease outlined herein. For example, the mixed form of uremic osteodystrophy consists of features typical of osteitis fibrosa; however, in addition, it is characterized by low forming activity with accumulation of excessive quantities of osteoid, as in osteomalacia. Another variant of mixed bone disease is similar to the aforementioned lesion, but the bone-forming parameters are normal. In the latter, the accumulation of osteoid is attributed to a shortage in the supply of mineral and hence delayed mineralization of organic bone matrix. This lesion responds favorably to vitamin D therapy.

TUMORAL CALCINOSIS

Extraskeletal calcification with periarticular, vascular, and other soft tissue calcium deposits is present in patients with chronic renal failure. They usually are associated with advanced secondary hyperparathyroidism featuring hyperphosphatemia with high calcium phosphate product.

Tumoral calcinosis in nonuremic patients with normal kidney, also known as hyperphosphatemic familial tumoral calcinosis, is a rare autosomal recessive disorder associated with large ectopic calcifications in soft tissue. Biochemical abnormalities include hyperphosphatemia secondary to increased tubular resorption of phosphorus and inappropriately normal or elevated $1,25(OH)_2D_3$. Recurrence of tumoral calcinosis after kidney transplantation suggests a deficiency of a systemic phosphaturic factor rather than an intrinsic tubular defect as an underlying mechanism (249).

Genetic analyses in patients with tumoral calcinosis identified three mutations that caused decreased bioactive circulating levels of FGF23 or end-organ resistance to FGF23. These mutations involve either genes encoding FGF23, or Klotho, or GA1Nac transferase 3 ($GALNT_3$). $GALNT_3$ is a Golgi-associated enzyme that o-glycosylates the furin-like convertase recognition sequence in FGF23. Mutations in FGF23 gene cause deficiency of FGF23 with renal phosphorus retention. $GALNT_3$ that selectively o-glycosylates the furin-like convertase recognition sequence in FGF23, thereby protecting it from proteolytic processing, is missing. This leads to low intact serum of FGF23 and high levels of inactive-terminal FGF23. Mutations in gene-coding Klotho decrease Klotho expression with a decreased level of FGF23-Klotho-FGF receptor complex leading to end-

organ resistance to FGF23. No effective treatment other than phosphorus restriction is available for tumoral calcinosis at this time.

CALCIFIC UREMIC ARTERIOLOPATHY (CALCIPHYLAXIS)

Calcific uremic arteriolopathy occurs most often in patients with end-stage renal disease who are undergoing dialysis or have recently received a kidney transplant. A similar abnormality has been associated with primary hyperparathyroidism, metastatic breast cancer, alcoholic liver cirrhosis, and Crohn disease. Calcific uremic arteriolopathy is characterized by small vessel calcifications with reduced perfusion and vascular thrombosis leading to acute infarction of subcutaneous fat tissue and cutaneous necrosis. The vascular lesions of small- to medium-sized subcutaneous arteries show calcification of the media. Some arteries are narrowed or occluded by intimal hyperplasia and fibrin thrombi. Interestingly, these changes are restricted to cutaneous arteries only. The reason for this anatomic predilection requires further investigation.

The diagnosis of calciphylaxis is suggested by characteristic features of the skin—painful pruritic skin lesion and subcutaneous nodules. These painful violaceous mottled lesions evolve into necrotic nonhealing ulcerations with gangrene formation. The legs are almost always involved. Severe proximal lesions on the legs and/or trunk are poor prognostic signs. The mortality rate is as high as 80%, principally because of secondary infection.

In 1962, Selye (250) described an animal model of calciphylaxis in which sensitization of rats with a systemic calcifying factor, such as high phosphate, high calcium diets, and PTH, and then challenge of the animals with trauma, iron salts, or albumin caused an acute local calcinosis followed by inflammation and sclerosis. However, absence of vascular calcifications in experimental calciphylaxis, as opposed to the vascular calcific lesions that are the hallmark of the lesions in uremic patients, led to the term “uremic calcific arteriolopathy” (UCA).

The pathogenesis and risk factors for UCA remain poorly understood. It has been proposed that the vascular smooth muscle cell may assume certain osteoblastic-like features and may play an active role in tissue calcification, including the production of bone matrix protein osteopontin. Indeed, blood vessels from the UCA lesions in patients stained positive for osteopontin and thus expressed an osseous protein (251). However, it is noteworthy that osteopontin, in fact, is an inhibitor of calcification, and the role of its presence in UCA vessels requires further evaluation. Whether

the vascular calcification is a passive or active process is still controversial (252). It is of importance that a new noninvasive imaging technology called electron-bean computed tomography has demonstrated that a high calcium phosphate product causes a progressive increase in calcium deposits in coronary arteries and in aortic and mitral valves in patients with advanced renal failure (253). Furthermore, hyperphosphatemia per se is an independent risk factor for high morbidity and mortality.

A recent study evaluating 19 patients with UCA in comparison with 54 patients without UCA with end-stage renal disease showed that hyperphosphatemia, high alkaline phosphatase level, low serum albumin concentration, and female gender appeared as highly significant risk factors for UCA (254). Other risk factors that were considered in the past included an elevated calcium phosphate product and high PTH. In this regard, parathyroidectomy was deemed as a potential therapeutic modality. In view of the new data, maintenance of normal serum phosphate level by aggressive control of hyperphosphatemia and maintenance of adequate nutritional status are important therapeutic measures. Vascular calcifications are associated with increased cardiovascular morbidity and mortality in patients with CKD. There are different types of cardiovascular calcifications. These include aging-associated medical sclerosis, lipid-associated calcifying atherosclerosis in the intima of vessel wall, calcifications of heart valves, and myocardial calcifications. Abnormalities in calcium and phosphate balance are well-known underlying factors involved in vascular calcifications. In addition to this is an imbalance in factors that antagonize calcifications such as Fetuin A or matrix GLa proteins, pyrophosphate, and magnesium. The latter two are small molecules. Furthermore, apoptosis of extracellular matrix protein and calciprotein particles may serve as nucleation factors for vascular calcification. It has been suggested, based on animal experiments, that vascular calcifications are an active process in which vascular smooth muscle cells transdifferentiate into osteogenic bone–forming cells. It has also been suggested that elevated phosphate levels could play a role in this transdifferentiation process.

A recent clinical study analyzed vascular calcifications in the breasts of CKD patients and found no evidence for osteogenic transdifferentiation. This important finding suggests that vascular calcification in patients with CKD is not a uniform biologic process and that different pathophysiologic mechanisms may mediate vascular calcifications in patients with CKD (255).

Vitamin K plays a major role in gamma-carboxylation of glutamate

(GLa) residues not only of coagulation factors but also of extrahepatic proteins involved in bone and vascular biology. The latter include bone-GLa-protein, also called osteocalcin (BGP), and matrix-GLa-protein (MGP). BGP knockout mice die from vascular calcifications. In many CKD patients, K levels are low. Low dietary K is associated with low bone density, fractures, and vascular calcifications (256).

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CHAPTER 7

Normal and Abnormal Magnesium Metabolism

Laurence Chan

Magnesium is the fourth most common cation in the human body, and it plays a critical role in many metabolic processes, including production and use of energy essential in the maintenance of normal intracellular electrolyte composition. Magnesium is necessary for a large number of enzymatic actions relating to the basic protein-synthesizing mechanisms. It maintains an important physiologic role particularly in cardiovascular and neuromuscular function. Approximately 60% of body magnesium is found in bone, and the remainder is found in cells. Only 1% is in extracellular fluid (ECF). As a result, the serum magnesium is a poor predictor of intracellular and total body stores and may grossly underestimate total magnesium deficit. Nevertheless, extracellular magnesium as measured in blood is broadly implicated in neuromuscular transmission and cardiovascular tone.

Overall, cellular and extracellular magnesium concentrations are carefully regulated by the gastrointestinal (GI) tract, kidney, and bone. GI losses and renal magnesium wasting constitute the major causes of magnesium deficiency. Several hereditary forms of hypomagnesemia have been discovered, including mutations in transient receptor potential melastatin type 6 (TRPM6), Claudin 16, Claudin 19, Cyclin M2 (CNNM2), and epidermal growth factor (EGF). Recently, mutations in magnesium transporter 1 (MagT1) were linked to T-cell deficiency

underlining the important role of magnesium in cell viability (1). Moreover, hypomagnesemia can be the consequence of the use of certain types of drugs, such as diuretics, EGF receptor inhibitors, calcineurin inhibitors, proton pump inhibitors, and antimicrobials. Magnesium supplementation, in addition to the use in hypomagnesemia, has been shown to be beneficial for preeclampsia, migraine, depression, coronary artery disease, and asthma. The therapeutic window of magnesium is wide, and in the absence of renal impairment, hypermagnesemia rarely occurs. Most cases of clinically significant hypermagnesemia are iatrogenic. Mild elevation in magnesium can occur in end-stage renal disease, tumor lysis syndrome, diabetic ketoacidosis, and in theophylline intoxication.

Normal Magnesium Metabolism

Magnesium is predominately an intracellular cation with less than 1% in the extracellular space. Owing to this distribution, total body magnesium concentrations are difficult to assess, and currently there is no simple accurate laboratory test to determine total body magnesium. The majority of our laboratory information comes from the determination of total magnesium in serum or plasma.

MEASUREMENT OF MAGNESIUM

Magnesium in serum or plasma can be found in three fractions: an ultrafilterable fraction consisting of ionized magnesium (70%–80%), complex-bound magnesium (1%–2%), and a protein-bound non-ultrafilterable fraction (20%–30%). In current clinical laboratories, magnesium is measured predominantly as plasma or serum concentration by autoanalyzer photometry using a chromatogenic reagent such as xylidyl blue. The color produced, measured bichromatically at 520/800 nm, is proportional to the magnesium concentration (2).

Serum magnesium concentrations can be reported as mEq/L, mmol/L, or mg/dL. One mEq/L = 0.5 mmol/L and is approximately 1.2 mg/dL. Serum magnesium in healthy persons is closely maintained within a normal range that varies between laboratories but is roughly 1.50 to 2.2 mEq/L (0.75–1.10 mmol/L). Since the molecular weight of magnesium is 24.3 and the valence of +2, a normal range of plasma magnesium concentration of 1.4 to 1.7 mEq/L is equivalent to 0.70 to 0.85 mmol/L or 1.7 to 2.1 mg/dL. Serum levels <1.5 mEq/L usually indicate magnesium

deficiency. When serum magnesium is between 1.5 and 1.7 mEq/L, a magnesium loading test can identify magnesium deficiency (1). A serum magnesium >2.2 mEq/L is diagnostic of hypermagnesemia.

Magnesium deficiency is commonly determined by measuring serum magnesium concentrations. However, serum magnesium values reflect only 1% of the body magnesium content, since most of the body's magnesium is stored in bone, muscle, and soft tissues. Therefore, the serum magnesium may grossly underestimate total magnesium deficits. Although serum values are within the normal range, the body can be in a severely magnesium-depleted state. Consequently, the clinical impact of magnesium deficiency may be largely underestimated. Only 20% of the serum magnesium is protein bound, in contrast to calcium, which is 40% bound to serum proteins. The variations in plasma protein concentration have less effect on serum magnesium than on calcium concentration.

Free magnesium in blood can be measured with an ion-sensitive electrode. The fraction of total magnesium bound to protein and other substances depends upon the pH. pH-dependency of ionized free magnesium (iMg^{2+}) in serum is expressed by the Siggaard-Andersen equation: iMg^{2+} (pH) = iMg^{2+} (7.4) × 10 × (7.4 − pH). During preparation of serum or plasma, considerable pH changes occur which have to be corrected on the basis of the above mentioned equation (2).

The total intracellular magnesium content approximates 8 to 10 mmol/L (10–20 mEq/L). However, most of the cell magnesium is bound to adenosine triphosphate (ATP) and other intracellular nucleotides, and in many enzyme complexes in which the Km is close to the free intracellular magnesium concentration. A number of techniques are available for the assay of cytosolic free magnesium. These include magnesium-selective electrodes, metallochromic indicators, P-31 nuclear magnetic resonance (NMR) spectroscopy, and fluorescent probes similar to those in the determination of cytosolic free calcium. Using magnesium-sensitive dyes based upon the FURA compound, the free cytosolic concentration is determined to be in the range of 0.6 to 0.8 mmol/L (1.2–1.6 mEq/L). However, there is some variation with cell type, and also some variation between regions of the cell. Intracellular magnesium concentrations can be determined non-invasively in vivo or in vitro by using NMR (3). The determination is based on the shift in the phosphorus (P-31) NMR spectrum of ATP, depending on the extent to which it is bound to magnesium. This technique is applicable to clinical studies in humans (4,5).

MAGNESIUM AND NUTRITION

The average daily diet in North America contains approximately 20 to 30 mEq (240–360 mg) of elemental magnesium. The requirement for magnesium is considered to be about 18 to 33 mEq/day for young men and 15 to 28 mEq/day for women. This suggests that the average western diet is only marginally adequate for maintenance of magnesium levels in healthy adults. Moreover, the requirements are higher during the rapid growth of infancy and adolescence as well as pregnancy and lactation. Magnesium is ubiquitous in our diet and is especially abundant in green vegetables rich in chlorophyll (a chelator of magnesium), as well as in seafood, grains, nuts, and meats (2). The United States NHANES 2005-2006 survey reported that more than half of all adults have an inadequate intake of magnesium (6). It has been suggested that a chronic magnesium deficiency (serum magnesium <0.75 mmol/L) is associated with an increased risk of many clinical conditions, including atherosclerosis, hypertension, cardiac arrhythmia, metabolic syndrome, type 2 diabetes mellitus, and insulin resistance.

MAGNESIUM HOMEOSTASIS

Under normal circumstances, the GI tract and kidney closely maintain magnesium balance (Fig. 7-1). It has been suggested that the minimum intake of magnesium required to maintain a positive balance in the body is approximately 0.3 mEq/kg/d.

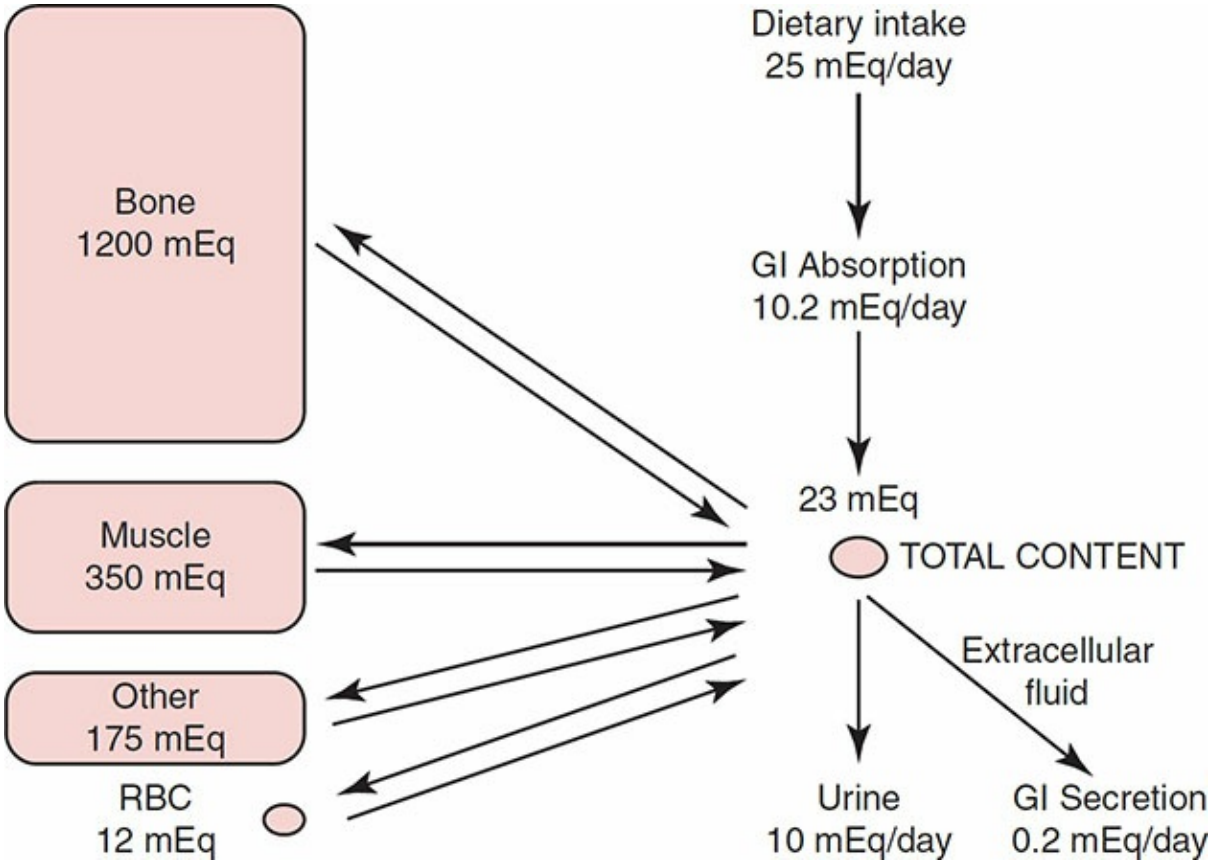

Figure 7–1 Schematic display of normal overall body homeostasis of magnesium, including an approximate distribution in different tissues. GI, gastrointestinal; RBC, red blood cells.

Only about 1% to 2% of the 21 to 28 g (1,750–2,400 mEq) of magnesium present in the adult human body is in the ECF compartment. The principal cellular stores of magnesium in the body are bone (67%) and muscle (20%) (Fig. 7-1).

Normal muscle has 76 mEq of magnesium/kg of fat-free solids, and much of this is complexed to intracellular organic phosphate and proteins (6). The normal magnesium level in red blood cells is about 4.6 mEq/L, of which 84% is thought to be complexed to ATP. The magnesium content of erythrocytes appears to be inversely related to the age of the cell, with the reticulocytes containing about two times more magnesium than older red blood cells. As noted, bone is the principal body store of magnesium. The normal calcium to magnesium ratio in bone is 50:1, with the ratio in trabecular bone being consistently higher than that in cortical bone. The major portion of magnesium is complexed with apatite crystal rather than bone matrix. Approximately 30% of bone magnesium is present as a surface-limited ion on the bone crystal and is freely exchangeable (7,8). However, considerable uncertainty exists with regard to the ease of

exchangeability of magnesium with its cellular source (7). Intracellular to extracellular distribution of magnesium is dissimilar to that of potassium. Minute changes in extracellular potassium rapidly result in changes in intracellular potassium concentration in muscle. Such shifts do not occur with magnesium because magnesium is bound to intracellular ligands and is not readily available for exchange in muscle. Less than 15% of muscle and erythrocyte magnesium is thought to be exchangeable (9).

In summary, bone and muscle cells are the major intracellular magnesium pools in humans, of which only a small fraction is exchangeable with the ECF. Magnesium balance is a function of intake and excretion. The average daily magnesium intake is 360 mg (15 mmol). Approximately one-third of this magnesium is absorbed, principally in the small bowel through both a saturable transport system mediated by a channel encoded by the *TRPM6* gene and passive diffusion. In the healthy adult, there is no net gain or loss of magnesium from bone so that balance is achieved by the urinary excretion of the approximately 100 mg (4.1 mmol) that is absorbed. Changes in intake are balanced by changes in urinary magnesium reabsorption, principally in the loop of Henle and the distal tubule

Gastrointestinal Absorption of Magnesium

About 30% to 40% of the normal dietary intake of magnesium is absorbed by the GI tract. The fraction of magnesium absorbed may increase to as high as 80% when the dietary magnesium intake is restricted to as low as 2 mEq/day and may decrease to 25% at high magnesium intakes of ≥45 mEq/day. Thus, magnesium absorption by the gut is nonlinear and varies inversely with intake. Magnesium absorption in humans and animals occurs primarily in the more distal portion of the small intestine, namely the jejunum and ileum (10). The small intestinal magnesium absorption appears to occur down an electrochemical gradient through a paracellular pathway.

In the colon transcellular absorption occurs. Magnesium crosses the brush border of the intestinal cell down an electrochemical gradient via the transient receptor potential melastatin channels TRPM6 and TRPM7 (1,11). The small intestinal paracellular pathway movement of magnesium occurs because of the positive magnesium chemical gradient across the paracellular channels. Magnesium absorption is also affected by paracellular water reabsorption. Bowel water absorption affects

magnesium concentration and absorption, and severe prolonged diarrhea results in intestinal secretion of magnesium.

The control of intestinal magnesium absorption is not well understood. Most studies have suggested that vitamin D has little effect on magnesium absorption (12). A study carried out in vitamin D-deficient patients showed that magnesium absorption was only minimally reduced before vitamin D repletion, and even following repletion it increased only slightly in contrast to the large change in calcium absorption (13). Similarly, Schmulen et al. (14) found that physiologic doses of 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$) normalized the modest defect in jejunal magnesium absorption in uremic patients. This might imply that although vitamin D may have a small effect on proximal absorption of magnesium, it has little effect on the more distal sites for magnesium absorption in the small bowel. Unlike in the kidney, the basic absorptive systems of calcium and magnesium are independent of each other in the intestinal tract; calcium flux is normally twice that of the magnesium flux at similar luminal concentrations.

It is probable that only ionized magnesium is available for absorption, and the amount available is affected by progressive precipitation of magnesium as insoluble phosphates, carbonates, and soaps beginning in the ileum, colon, and (ultimately) stool. Alterations of luminal concentrations of calcium and phosphate also indirectly affect magnesium absorption. Conversely, the elevation of intraluminal magnesium concentration may precipitate phosphate and thereby allow for greater calcium absorption. Steatorrhea may potentiate magnesium malabsorption through the formation of nonabsorbable magnesium lipid salts (15).

The major portion of magnesium found in the stool is derived from the diet. The magnesium concentration in saliva, gastric secretions, bile, and pancreatic and intestinal secretions ranges from 0.3 to 0.7 mmol and amounts to only about 1% of the daily fecal output. Taken together, overall knowledge of the precise control and regulation of magnesium absorption in the intestinal tract is still lacking.

Genetic screenings and microarray-based expression studies have resulted in the identification of numerous magnesium-transporting proteins (Table 7-1). Our knowledge of renal magnesium transport has increased dramatically due to the discovery of the molecules involved in renal magnesium handling from the study of rare human genetic conditions (Table 7-2).

The understanding of these genetic conditions coupled with drug-induced disorder of magnesium homeostasis has enhanced our knowledge

of normal and abnormal magnesium metabolism.

Table 7–1 Magnesium Transporters

Name	Membrane	Expression	Mechanism
General Magnesium Transporters			
TRPM7	Plasma membrane	Ubiquitous	Channel
MagT1	Plasma membrane	Ubiquitous	Channel
SLC41A1	Plasma membrane	Ubiquitous	Exchanger
SLC41A2	Golgi membrane	Ubiquitous	Exchanger
CNNM3	Plasma membrane	Ubiquitous	Transporter
MRS2	Mitochondrial membrane	Ubiquitous	Channel
Tissue-Specific Magnesium Transporters			
TRPM6	Apical plasma membrane	Kidney, intestine	Channel
CNNM2	Basolateral membrane	Kidney	Transporter/Sensor
CNNM4	Basolateral membrane	Intestine	Exchanger(Mg extrusion)

MagT1, magnesium transporter 1; SLC41, solute carrier family 41 member, A1 and A2; function as Na/Mg exchanger; TRPM6, transient receptor potential melastatin type 6; TRPM7, transient receptor potential melastatin type 7; CNNM2, Cyclin M2; member of the Cyclin M family; function as magnesium transporter; CNNM4, Cyclin M4; member of the Cyclin M family.

Table 7–2 Genetic Causes of Hypomagnesemia

Gene	Protein	Disease	Inheritance	Segment	Blood Mg^{2+}	Urine Mg^{2+}	Blood Ca^{2+}	Urine Ca^{2+}	Other Symptoms
CLDN16	Claudin 16	FHHNC type 1	R	TAL	↓		–		Nephrocalcinosis, renal failure
CLDN19	Claudin 19	FHHNC type 2	R	TAL	↓		–		Nephrocalcinosis, renal failure, visual impairment
SLC12A1	NKCC2	Bartter type 1	R	TAL	↓	–	–		Na^+ wasting, hypokalemic alkalosis, high renin/aldosterone
KCNJ1	ROMK	Bartter type 2	R	TAL	↓	–	–		Na^+ wasting, hypokalemic alkalosis, high renin/aldosterone
CLCNKB	ClC-Kb	Bartter type 3	R	TAL	↓	–	–		Na+ wasting, hypokalemic alkalosis, high renin/aldosterone
BSND	Barttin	Bartter type 4	R	TAL	↓	–	–		Na+ wasting, hypokalemic alkalosis, high renin/aldosterone
TRPM6	TRPM6	HSH	R	DCT	↓	–	↓	–	Seizures, muscle spasms, mental retardation
EGF	EGF	IRH	R	DCT	↓		–	–	Seizures, mental retardation
CNNM2	CNNM2	HSMR	D/R	DCT	↓	–	–	–	Seizures, mental retardation
KCNA1	Kv1.1	ADH	D	DCT	↓	–	–	–	Muscle cramps, tetany, myokymia
KCNJ10	Kir4.1	SeSAME/ EAST	R	DCT	↓		–	↓	Hypokalemia, metabolic alkalosis, sensorineural deafness, seizures, ataxia, mental retardation
FXYD2	FXYD2	IDH	D	DCT	↓		–	↓	Convulsions
HNF1B	HNF1β	RCAD	D	DCT	↓		–	↓	Renal cysts, MODY5, renal malformations
PCBD1	PCBD1	RCAD-like	R	DCT	↓		–	–	Transient hyperphenylalaninemia, MODY5-like
SLC12A3	NCC	Gitelman syndrome	R	DCT	↓		–	↓	Hypokalemia, metabolic alkalosis, tetany, chondrocalcinosis

SLC, solute carrier; NKCC2, $Na^+/K^+/2Cl^-$ cotransporter; TRPM6, transient receptor potential melastatin type 6; EGF, epidermal growth factor; CNNM2, Cyclin M2; FXYD2, FXYD domain containing ion transport regulator 2; HNF1B, hepatocyte nuclear factor 1B; PCBD1, pterin-4-alpha-carbinolamine dehydratase; NCC, Na^+/Cl^- cotransporter; FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HSH, hypomagnesemia with secondary hypocalcemia; IRH, isolated recessive hypomagnesemia; HSMR, hypomagnesemia with seizures and mental retardation; ADH, autosomal dominant hypomagnesemia; SeSAME, sensorineural deafness, seizures, ataxia, mental retardation, and electrolyte imbalance; EAST, epilepsy, ataxia, sensorineural deafness, and tubulopathy; IDH, isolated dominant hypomagnesemia; RCAD, renal cysts and diabetes; D, dominant; R, recessive; TAL, thick ascending limb of Henle's loop; DCT, distal convoluted tubule; MODY, maturity-onset diabetes of the young.

Renal Excretion of Magnesium

The status of body magnesium balance and particularly ECF magnesium concentration is largely determined by the renal excretion of magnesium. The main determinant of magnesium balance is the serum magnesium concentration itself, which directly influences renal excretion. Hypomagnesemia stimulates tubular reabsorption of magnesium, whereas hypermagnesemia inhibits it.

On a normal dietary intake of magnesium, urinary magnesium excretion averages 100 to 150 mg/day or 8 to 12 mEq/day. In patients receiving supplementary oral magnesium-containing antacids, urinary magnesium excretion can increase to 500 to 600 mg/day or more with little change in serum magnesium levels. Similarly, when dietary magnesium restrictions are imposed, 24-hour urinary magnesium excretion decreases in 4 to 6 days to as low as 10 to 12 mg (1 mEq) (16). Thus, the ability of the kidney to conserve magnesium is excellent when it is needed.

In chronic kidney disease (CKD) the fractional excretion of magnesium rises sharply as glomerular filtration rate progressively falls, thus protecting against the development of significant hypermagnesemia. Urinary magnesium excretion can approximate the filtered load of magnesium with marked hypermagnesemia secondary to high dietary intake or intravenous magnesium infusion. Studies in several species have shown that there is a threshold value for magnesium excretion, close to the normal magnesium concentration (17). Thus, when serum magnesium concentration falls slightly, urinary magnesium excretion rapidly decreases to very low values. Conversely, when serum magnesium rises slightly above normal, magnesium excretion rapidly increases.

GLOMERULAR FILTRATION

Approximately 70% of plasma magnesium is in the ionic form with the remaining 30% bound to plasma protein. As a result, about 70% to 80% of plasma magnesium is freely filtered at the glomerulus.

PROXIMAL TUBULE

Magnesium transport differs from that of most other ions in that the proximal tubule is not the major site of reabsorption. Fractional magnesium absorption is substantially less than that of sodium or calcium. Only 15% to 25% of the ultrafilterable magnesium is reabsorbed passively

in the proximal tubule. Luminal magnesium concentrations rise along the length of the proximal convoluted tubule to a value as high as 1.5 times greater than that of the ultrafilterable magnesium in glomerular filtrate (Fig. 7-2) (18). The major influence on proximal magnesium reabsorption is the status of the ECF volume. Absorption is enhanced in states of volume depletion, whereas absorption is decreased in volume-expanded states.

LOOP OF HENLE

The early micropuncture studies of Morel et al. (18) indicated that, unlike most cations, the loop of Henle is the major site of magnesium reabsorption. Magnesium concentration in the early distal tubule fluid is only 60% to 70% of the ultrafilterable magnesium concentration, suggesting that some 50% to 60% of the filtered magnesium is reabsorbed in the loop of Henle, primarily in the thick ascending limb (Fig. 7-2).

Magnesium absorption in the loop of Henle is passive via a paracellular pathway and dependent on the transepithelial voltage gradient partly generated by a sodium back-leak into the lumen via the paracellular protein Claudin 16 (19) (Fig. 7-3).

In hypermagnesemic states, magnesium reabsorption in the loop of Henle approaches zero (20). Conversely, in hypomagnesemic states, the loop of Henle more avidly reabsorbs magnesium, allowing only minimal amounts, <3%, of the filtered load to reach the distal tubules and be excreted in the urine (21).

An important interaction between calcium and magnesium has been observed in the thick ascending limb. It is well known that hypercalcemia (20) or hypermagnesemia inhibits both magnesium and calcium reabsorption (21,22). Studies show that this effect is mediated by the calcium-sensing receptor present on the basolateral membrane of the thick ascending limb and distal collecting tubule, which modulates absorption by changes in plasma divalent cation concentrations (23,24).

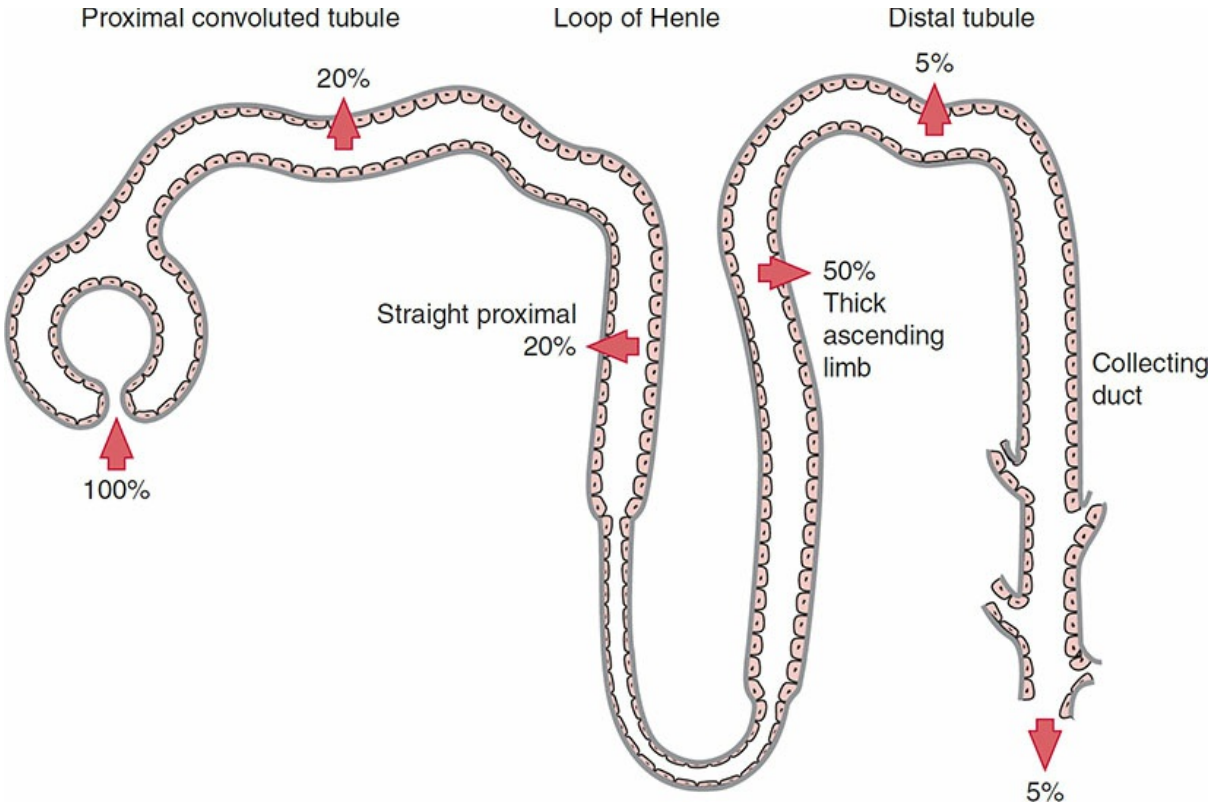

Figure 7–2 Normal distribution of magnesium reabsorption as a percentage of the ultrafilterable magnesium at the glomerulus.

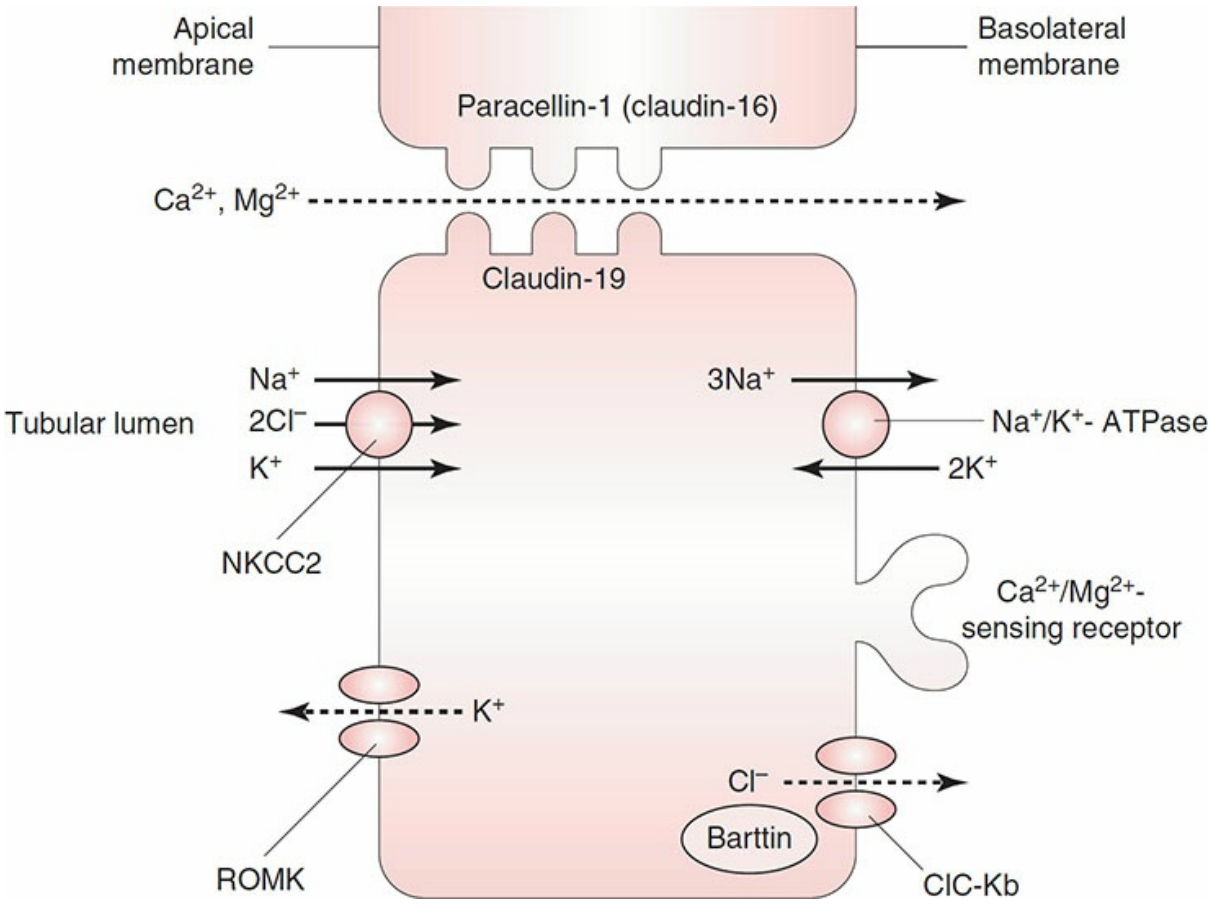

Figure 7–3 A model of magnesium transport mechanisms in the hick ascending limb of Henle's loop (TAL). Magnesium absorption is paracellular, driven by the electrochemical gradient partly generated by a complex of Claudin-16 and Claudin-19. Sodium is reabsorbed via the apical Na/K/2Cl cotransporter (NKCC2), and pumped out basolaterally by the Na/K ATPase. Chloride exits via the chloride channel composed of chloride channel Kb (ClC-Kb) and Barttin. Potassium is recycled apically via the inwardly rectifying K channel (ROMK), thereby generating a lumen-positive transtubular voltage. The Ca/Mg sensing receptor also plays a role in magnesium absorption.

DISTAL REABSORPTION

In the distal convoluted tubule, fine-tuning of magnesium reabsorption occurs. Genetic diseases that produce hypomagnesemia have helped elucidate important components of distal magnesium transport. Magnesium transport in the distal tubule is active and transcellular, with reabsorption occurring via an apical transient receptor potential channel, melastatin subtype 6 (TRPM6) (19,25) (Fig. 7-4).

The mechanisms for cytoplasmic diffusion and basolateral transport are not yet known (26). Sex hormones, acid–base status, and peptide hormones such as calcitonin, glucagon, arginine, vasopressin, and parathyroid hormone (PTH) enhance magnesium uptake in distal convoluted tubule cells (23,26,27) possibly via modulation of TRPM6. Mutations in TRPM6 cause autosomal recessive hypomagnesemia with secondary hypocalcemia (HSH), which is due both to impaired intestinal absorption of magnesium and a renal magnesium leak.

Recently, EGF has been demonstrated to be a magnesiotropic hormone directly stimulating TRPM6 activity (28). This explains the finding that magnesium wasting occurs in patients with colorectal cancer who are treated with cetuximab, an EGF receptor (EGFR)-targeted monoclonal antibody that prevents receptor stimulation (29).

Furthermore, both tacrolimus and cyclosporine A decrease TRPM6 expression, possibly explaining the hypomagnesemia seen in patients treated with these medications (25).

Physiologic and Pharmacologic Effects

The importance of magnesium in the body can be traced back in history. The first documented use of magnesium in medicine was in 1697 when Dr. Grew identified magnesium sulfate as the major ingredient of Epsom salt

which was extracted from a well in Epsom, England, and was used over the years to treat abdominal pain, constipation, muscle strains, and cerebral edema (30). The chemistry of magnesium and other alkali metals was characterized by Humphrey Davy in 1808 (31) and the first determination of the element was in plasma in 1920 (32). Magnesium plays a critical and necessary role in intracellular metabolism. Magnesium is necessary for a wide spectrum of enzymatic reactions, including various phosphokinases and phosphatases (33), which are involved in energy storage and use. Phosphatases are particularly important because magnesium functions primarily to form magnesium ATP, which is a substrate for these enzymes. These ion-sensitive ATPases are situated in the intracellular compartments and membranes to regulate the flow of potential energy from the mitochondria and cytoplasm. Recognized magnesium ATPases include ouabain-sensitive $Mg^{2+}/Na^{+}/K^{+}$ATPase, ouabain-insensitive Mg^{2+}, $HHCO_3$ATPase, and Mg^{2+}/Ca^{2+}ATPase, which are associated with the sodium, proton, and calcium pumps, respectively. They are all essential for ionic control of the cell composition (34). Magnesium also is involved in protein synthesis through its action on nucleic acid polymerization, its role in ribosomal binding to ribonucleic acid (RNA), and in the synthesis and degradation of deoxyribonucleic acid (DNA). In addition to its role in phosphorylation of glucose, magnesium may also control mitochondrial oxidative metabolism (35). Adenylate cyclase, critical in the generation of the intracellular secondary messenger 3′,5′-cyclic adenosine monophosphate (cAMP), also has been shown to be dependent on magnesium (36). Intracellular magnesium has also been shown to have an important regulatory function on both K^{+} and Ca^{2+} channels (37).

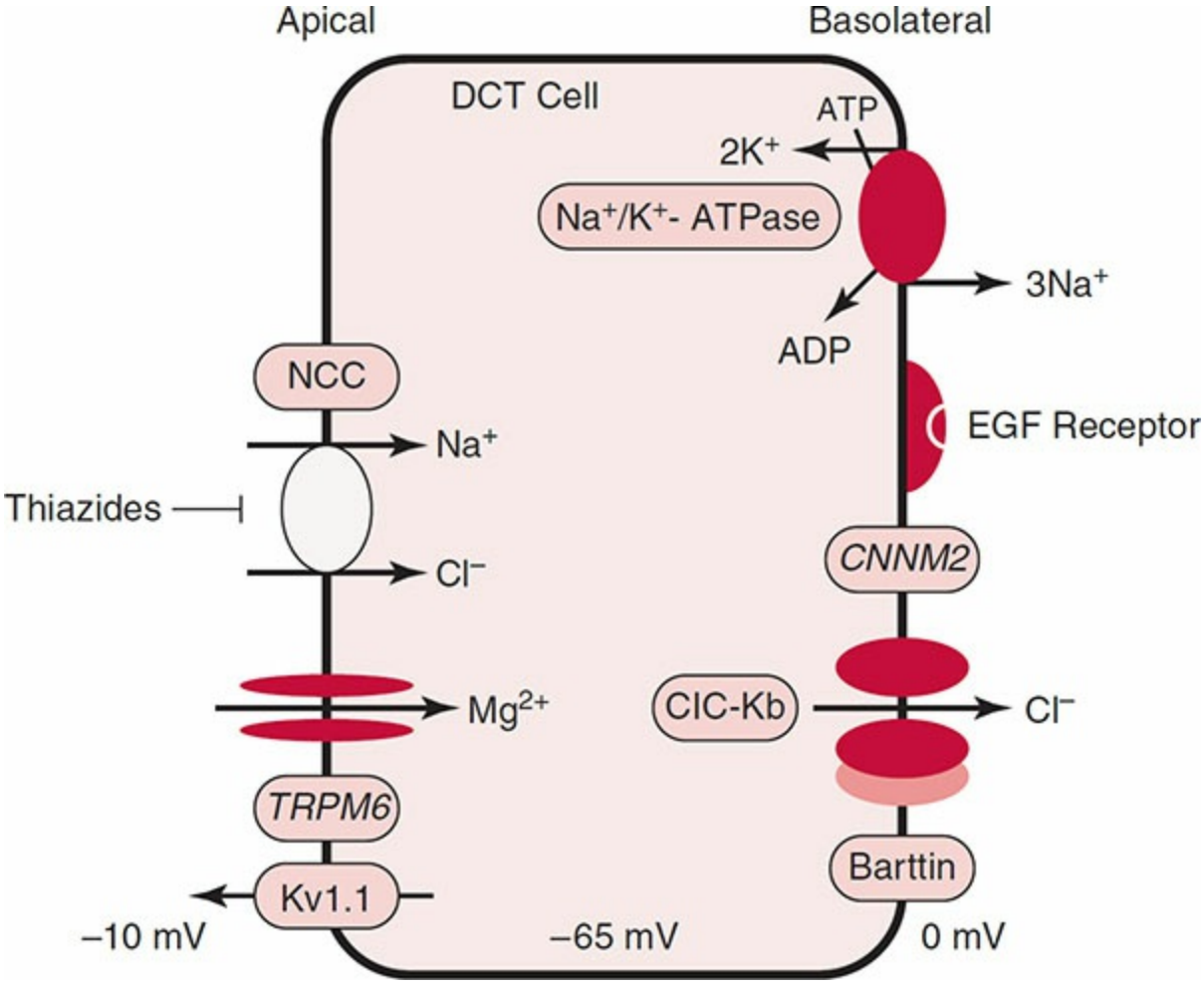

Figure 7–4 A model of magnesium transport mechanisms in the DCT. Magnesium is transcellular via the recently identified magnesium channel *TRPM6* in the apical membrane. It is driven by the hyperpolarized membrane potential generated by the apical potassium channel, Kv1.1. Basolateral magnesium exit may occur through Cyclin M2 (*CNNM2*), which acts as a magnesium channel or transporter. The link between the defect in the apical thiazide-sensitive NCC seen in Gitelman syndrome or the basolateral chloride channel (ClC-Kb) defect seen in Bartter syndrome with sensorineural deafness has not been clearly identified. The basolateral EGF receptor that modulates the apical *TRPM6* magnesium channel is also depicted. NCC, NaCl cotransporter; DCT, distal convoluted tubule; Kv1.1, voltage-gated potassium channel 1.1; NCC, sodium chloride cotransporter; *TRPM6*, transient receptor potential melastatin type 6.

Intracellular magnesium is found in the free-ion form or complexed to proteins or organophosphates. The free magnesium concentration determines the effect of concentrations of the high-energy nucleotide complex magnesium ATP. Knowledge of the true ionic concentration of magnesium in the cell is important but difficult to measure. It is thought that only about 5% to 15% of cellular magnesium is truly ionized (38).

Magnesium Deficiency

Because magnesium is an essential element for both plants and animals, it exists almost everywhere in our environment. Thus, it is rare to see spontaneous magnesium depletion from dietary indiscretion unless it is severe. Still, it has been suggested that the normal dietary intake of magnesium is marginal. The first description of symptoms related to hypomagnesemia was in 1960 when Vallee et al. (39) described five patients with hypomagnesemia and symptoms and signs that are now felt to be classic for magnesium depletion. These patients had carpopedal spasms with positive Chvostek and Trousseau signs, and three of the five subjects also had convulsions. All patients' symptoms and signs abated following magnesium administration. Several investigators have attempted to produce magnesium depletion in humans by placing subjects on a low magnesium intake. In most studies, only minimal magnesium depletion has been induced. Shils (40), however, was able to cause severe magnesium depletion in seven patients who were placed on diets extremely low in magnesium for an extended period of time. Symptoms that appeared to be related to magnesium depletion developed in six of the seven patients; five had a positive Trousseau sign, and two of these patients also had a positive Chvostek sign. All patients became lethargic and showed generalized weakness, anorexia, nausea, and apathy. Biochemical abnormalities included hypomagnesemia, hypocalcemia, hypokalemia, and decreased total body exchangeable potassium. All abnormalities reverted to normal with replacement of magnesium alone.

Clinical Conditions Associated with Magnesium Depletion

DEFECTIVE GASTROINTESTINAL ABSORPTION OF MAGNESIUM

Gastrointestinal causes of magnesium depletion can be divided into four categories: decreased intake, steatorrheic states, severe diarrheal states, and selective magnesium malabsorption (Table 7-3). Caddell and Goddard (41) found serum magnesium to be subnormal in 19 of 28 children with protein calorie malnutrition (kwashiorkor). This was felt to have resulted from the combination of poor intake of magnesium, vomiting, and

diarrhea. Similarly, magnesium depletion has been described in hospitalized patients who have been maintained on parenteral nutrition for prolonged periods of time (42,43).

Steatorrheic State

Hypomagnesemia has been described in a number of patients with small bowel disease. Booth et al. (15) found that 15 of 42 patients with malabsorption syndromes had subnormal serum magnesium levels. They were able to show a rough correlation between serum magnesium levels and the degree of steatorrhea, suggesting that the magnesium malabsorption might be a consequence of the formation of insoluble magnesium soaps. Supporting this possibility is the finding that magnesium absorption was improved when the patients were placed on a low-fat diet. The small bowel diseases with the highest incidence of hypomagnesemia are idiopathic steatorrhea and disease of the distal ileum.

Table 7–3 Causes of Hypomagnesemia

Gastrointestinal
Inadequate intake
Steatorrheic states
Severe diarrhea
HSH (renal defect also exists)
Renal
Intrinsic
Inherited disorders
FHHNC: type 1 due to Claudin 16 mutation and type 2 due to Claudin 19 mutation
Autosomal dominant hypomagnesemia with hypocalciuria
Autosomal dominant isolated hypomagnesemia (Na/K ATPase gamma subunit, Kv1.1 and Cyclin M2 mutations)
Autosomal recessive isolated hypomagnesemia (EGF mutation)
Renal malformations and early-onset diabetes mellitus (HNF1-B mutation)
HSH (main defect may be GI)
ADH
IRH
Gitelman syndrome
Bartter syndrome
Acquired disorders

Antimicrobials: Aminoglycosides, pentamidine, amphotericin B, Foscarnet *cis*-Diaminedichloro platinum Cyclosporine/tacrolimus; rapamycin Proton pump inhibitors EGFR inhibitors: Cetuximab, panitumumab, matuzumab Diuretics: Furosemide; Thiazide
Extrinsic (intrarenal)
Volume expansion Hypercalciuria Sodium loads Diabetic ketoacidosis Diuretics Hyperaldosteronism Antidiuretic hormone secretion syndrome
Miscellaneous
Alcoholism Thyrotoxicosis Burns Hungry bone syndrome

ADH, autosomal dominant hypocalcemia; FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HSH, hypomagnesemia with secondary hypocalcemia; IRH, isolated recessive hypocalcemia.

Diarrheal States

Besides the steatorrheic states, magnesium depletion can occur in any severe diarrheal state (44,45). As with potassium, fecal magnesium excretion is related to the total water content where the stool magnesium concentration is approximately 6 mEq/L (45). Magnesium depletion also has been described in patients following jejunoileal bypass surgery for the treatment of morbid obesity, probably from a combination of factors, including malabsorption, shortened transit time, and diarrhea (46).

Hereditary Magnesium Absorptive Defect

Several patients have been described who have a defect in the GI absorption of magnesium (47–49). In this disorder of HSH, profound hypomagnesemia develops in the first few months of life. The absorptive defect can be overcome, however, by an oral intake of high-dose

magnesium. Most of these patients have severe hypomagnesemia, hypocalcemia, tetany, and seizures. The defect is an autosomal recessive disorder and results in the failure of active transcellular magnesium absorption. It is due to a mutation in the *TRPM6* gene, which encodes for the apical membrane TRPM6 channel in the intestine and the distal convoluted tubule (50,51) (Table 7-1).

RENAL MAGNESIUM WASTING

Renal magnesium wasting can be of two distinct types. One represents a primary kidney defect, whereas the second represents the kidney's normal response to a variety of systemic and local factors that increase magnesium losses (Table 7-3). Symptomatic hypomagnesemia is much more likely to be seen in the state of primary renal magnesium wasting. The hallmark of both of these states is a disproportionately elevated urinary magnesium excretion in association with hypomagnesemia. Normally, when serum magnesium falls only slightly owing to extrarenal causes, urinary magnesium falls to <1 mEq/day (12 mg/day), whereas if the kidney is responsible for the magnesium losses, urinary magnesium is increased relative to the hypomagnesemic state (>4 mEq/day). Thus, urinary magnesium should be measured before magnesium replacement to determine whether the hypomagnesemic state resulted from renal or extrarenal causes.

Primary Renal Magnesium Wasting

Primary renal magnesium wasting can occur from either inherited or acquired causes. A number of inherited forms of renal magnesium wasting have been described recently (Table 7-2).

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis

The most severe form of renal magnesium wasting results from an impairment of tubular reabsorption of magnesium and calcium in the thick ascending limb of Henle's loop (52,53). This autosomal recessive disorder known as familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) (1,11) results from mutations in the *CLDN16* gene, which encodes the renal tight junction protein Claudin-16 (formally paracellin-1) (Fig. 7-3). This protein is thought to allow selective back-leak of sodium over chloride into the tubule lumen, enhancing the driving

force for paracellular magnesium reabsorption (11). Clinical and laboratory features of FHHNC include presentation at a young age with nephrocalcinosis, polyuria, CKD, hypomagnesemia, and increased urinary magnesium and calcium excretion. Hypomagnesemia is unresponsive to magnesium replacement, and progression to end-stage renal disease is common. FHHNC may occasionally be due to mutation in Claudin-19. In addition to hypomagnesemia, and nephrolithiasis, such patients have ocular involvement with macular colobomata, nystagmus, and myopia.

Na/K ATPase Mutation

An autosomal dominate disorder of hypomagnesemia with hypocalcemia has been linked to a heterozygous mutation of FXYD2 on chromosome 11q23 (54). This encodes for a γ subunit of the basolateral Na^+/K^+ ATPase. The mutation results in misrouting of this subunit and defective magnesium reabsorption in the distal convoluted tubule where this subunit is normally expressed in the basolateral membrane (55) (Fig. 7-4).

Epidermal Growth Factor Gene Mutation

Isolated recessive hypomagnesemia (IRH) is a rare disorder due to a mutation of the EGF precursor protein. EGF is a magnesiotropic hormone that stimulates TRPM6, an apical magnesium channel in the distal convoluted tubule. The defect in this gene results in decreased TRPM6-mediated apical magnesium uptake in the distal convoluted tubule and magnesium wasting (19,28) (Fig. 7-4).

Voltage-Gated Potassium Channel

A mutation in the gene KCNA1 that encodes the voltage-gated potassium channel Kv1.1 is the cause of isolated autosomal dominant hypomagnesemia discovered in a large Brazilian family. This channel colocalizes with the magnesium channel TRPM6 in the distal collecting tubules. Wild-type Kv1.1 appears to control TRPM6 magnesium reabsorption via the creation of an appropriate potential across the luminal membrane (1).

Hepatocyte Nuclear Factor-1-β Gene Mutations

Hypomagnesemia and renal magnesium wasting have been reported in 8 of

18 patients (44%) with known mutations in the hepatocyte nuclear factor-1-β (HNF-1-β) gene. HNF-1-β is a transcription factor that regulates the expression of the gamma subunit of Na/K ATPase. These findings suggest that some mutations of HNF-1-β can activate this subunit, thereby causing hypomagnesemia, in association with early-onset diabetes and renal malformations (1,56).

Cyclin M2 Mutations

Mutations in the Cyclin M2 (*CNNM2*) gene have been implicated in families with dominant isolated renal magnesium wasting. CNNM2 encodes a transmembrane protein that is localized to the basolateral membrane of the thick ascending limb and distal convoluted tubule (1).

Autosomal Dominant Hypocalcemia

Activating mutations in the calcium-sensing receptor located in the parathyroid chief cell and basolateral membrane of the thick ascending limb of the loop of Henle and distal convoluted tubule results in autosomal dominant hypocalcemia (ADH). The defect results in decreased kidney calcium and magnesium reabsorption and often hypomagnesemia (57).

Gitelman Syndrome

Gitelman syndrome is caused by a heterogeneous group of loss-of-function mutations usually in the solute carrier family 12, member 3 gene, *SLC12A3*, which encodes the thiazide-sensitive NaCl cotransporter (NCC) (52) (Fig. 7-3). In a minority of patients the mutation is in the *CLCNKB* gene encoding the chloride channel CID-Kb, the same gene mutation defect found in Bartter syndrome with sensorineural deafness suggesting a highly variable phenotype in patients with *CLCNKB* mutations (58). Gitelman syndrome is an autosomal recessive heritable kidney disease characterized by hypomagnesemia, hypocalciuria, and hypokalemia metabolic alkalosis. This syndrome occurs in an older age group and usually has mild clinical symptoms, although patients may complain of musculoskeletal cramps, muscle weakness, muscle stiffness, arthralgias, nocturia, polydipsia, and thirst (57,58). The mechanism for the moderate renal magnesium wasting in Gitelman syndrome has not been definitively characterized (58) but may relate to reduced abundance of the TRPM6 epithelial magnesium channels in the distal convoluted tubule (59).

Bartter Syndrome

Bartter syndrome, a defect in the chloride channel of the thick ascending limb cells, also may be associated with mild hypomagnesemia. Several different basolateral chloride channels play a role in chloride reabsorption; hence different clinical subtypes of Bartter syndrome were described (Table 7-4). In contrast to Gitelman syndrome, Bartter syndrome occurs at a younger age. It often presents in childhood and may be associated with growth and mental retardation, hypokalemia, and metabolic alkalosis. Polyuria and polydipsia are present due to decreased urinary concentrating ability (59) (Table 7-4).

Drug-Induced Renal Magnesium Wasting

The acquired forms of renal magnesium wasting are largely drug induced. Osmotic diuretics such as mannitol increase urinary magnesium excretion. Both loop diuretics and thiazide can inhibit net magnesium reabsorption, while potassium-sparing diuretics may enhance magnesium transport and lower magnesium excretion. Renal magnesium wasting has been well documented in a number of patients receiving aminoglycosides (60,61). Renal magnesium wasting also has been described in patients receiving *cis*-diaminedichloro platinum (*cis*-DDP) (62). In one series, 23 of 44 patients treated with *cis*-DDP developed hypomagnesemia. Two of these patients required hospitalization because of severe symptomatic magnesium depletion. In an additional report of 50 patients treated with multiple courses of combined chemotherapy with *cis*-DDP, 76% developed hypomagnesemia (63). This defect in renal magnesium handling can persist for months after the *cis*-DDP has been discontinued (63,64).

Table 7–4 Gitelman and Bartter Syndrome

Disorder	Gene Affected	Gene Product	Clinical Presentation	Functional Studies
Gitelman syndrome	SLC12A3	NCCT	Gitelman syndrome	Concentrating capacity normal/near normal and diluting capacity

				reduced
Bartter syndrome type I	SLC12A1	NKCC2	Antenatal Bartter syndrome (hyperprostaglandin E syndrome)	Concentrating capacity reduced and diluting capacity reduced
Bartter syndrome type II	KCNJ1	ROMK	Antenatal Bartter syndrome	Concentrating capacity reduced and diluting capacity reduced
Bartter syndrome type III	CLCKB	CLC-Kb	Classical Bartter syndrome	Concentrating capacity reduced and diluting capacity reduced
Bartter syndrome type IV	BSND	Barttin (B-subunit of CLC-Ka and CLC-Kb)	Antenatal Bartter syndrome (hyperprostaglandin E syndrome) and sensorineural deafness[a]	Concentrating capacity reduced and diluting capacity reduced
Bartter syndrome type IVB	CLCNKA and CLCNKB	CLC-Ka and CLC-Kb	Antenatal Bartter syndrome (hyperprostaglandin E syndrome) and sensorineural deafness[a]	Concentrating capacity reduced and diluting capacity reduced
Bartter syndrome type V	CaSR	CaSR	Bartter syndrome with hypocalcemia	Concentrating capacity reduced and diluting capacity reduced

Genetics and presentation of Bartter and Gitelman syndromes. There are six Bartter

syndrome subtypes (I, II, III, IV, IVB, and V) corresponding to six genetic defects. NKCC2, furosemide-sensitive sodium-potassium-2 chloride cotransporter; ROMK, renal outer medullary potassium channel; CLC-Kb, chloride channel Kb; CLC-Ka, chloride channel Ka; CaSR, calcium-sensing receptor; NCCT, thiazide-sensitive sodium chloride cotransporter.

[a]Sensorineural deafness occurs because ClC-Ka and CLC-Kb are highly expressed in the inner ear and interact with other transport proteins (e.g., NKCC1) to maintain the high potassium concentration in the endolymph that is required for normal hearing. Some experts classify the mild salt-losing effect of gain-of-function mutations in the calcium-sensing receptor as Bartter syndrome type V.

Hypomagnesemia is common in patients who have received solid organ transplants and may be an important risk factor for new-onset diabetes mellitus after transplantation. The calcineurin inhibitors, cyclosporine and tacrolimus, are drugs that commonly cause hypomagnesemia (65) possibly by decreasing TRPM6 expression, resulting in renal magnesium wasting. These drugs may also increase Claudin-14 expression which would inhibit paracellular magnesium transport. In contrast to other hypomagnesemic states, which are usually accompanied by hypokalemia, cyclosporine/tacrolimus-induced hypomagnesemia usually is associated with either normokalemia or hyperkalemia at times. Proton pump inhibitors (66), commonly used in transplant recipients, can also cause hypomagnesemia due to the loss of active magnesium absorption via transient receptor potential melastatin-6 and -7 (TRPM6 and TRPM7) (Table 7-1). The hypomagnesemia rapidly abates on discontinuation of these drugs.

Renal magnesium wasting has been described in patients with acquired immunodeficiency syndrome undergoing treatment with pentamidine for *Pneumocystis carinii* pneumonia (67). Symptomatic hypomagnesemia can develop up to 2 weeks after cessation of therapy with this agent. In addition, hypomagnesemia has been reported in patients receiving foscarnet, an antiviral agent used to treat cytomegalovirus disease (68), and amphotericin B treatment of fungi infection (69).

Magnesium wasting occurs in patients with colorectal cancer who are treated with cetuximab, an EGFR-targeted monoclonal antibody that prevents receptor stimulation (70). As EGF has been demonstrated to be a magnesiotropic hormone directly stimulating TRPM6 activity, blockade of this receptor simulation results in magnesium wasting (28). A similar side effect can also occur in other antibodies targeting EGF receptor including panitumumab and matuzmab.

Secondary Forms of Renal Magnesium Wasting

Tubular reabsorption of magnesium is linked to a variety of other cations. Both sodium and calcium infusions can markedly increase urinary magnesium (71). Usually because of the modest nature and short duration of infusions, magnesium depletion does not develop. However, hypomagnesemia may develop when large saline infusions are given in association with diuretics, as are used in the treatment of hypercalcemia. Any chronic hypercalciuric state, such as seen with vitamin D therapy, also can cause magnesium wasting (72,73).

Virtually all diuretics, with the exception of acetazolamide, can increase magnesium excretion but only modestly, and magnesium supplementation usually is not required.

Hypomagnesemia secondary to starvation was first recognized during World War II (74). Jones et al. (75) subsequently showed that patients undergoing total starvation had an average magnesium loss of 10 mEq/day in their urine and suggested that ketoacidosis was responsible for this loss. Similarly, in patients with untreated diabetic ketoacidosis, there is marked renal magnesium wasting in the acidotic period as well as during early treatment (76). Following insulin and fluid therapy, serum magnesium may fall precipitously and tetany may occur (76,77). Phosphate replacement therapy in patients with diabetic ketoacidosis also has been associated with the induction of hypomagnesemia (78). In view of these findings, some (79) have suggested that in addition to the other cations commonly given, magnesium replacement should be included in the management of diabetic ketoacidosis. However, the American Diabetic Association recommends magnesium supplementation only in those patients who have documented hypomagnesemia (79).

Increased urinary magnesium excretion also has been reported with primary and secondary hyperaldosteronism (80,81) and inappropriate secretion of antidiuretic hormone (82). The renal magnesium wasting in these settings, however, is usually not severe enough to cause clinically significant magnesium depletion.

MISCELLANEOUS CAUSES OF HYPOMAGNESEMIA

Hypomagnesemia has been a common finding in patients with chronic alcoholism (82). Ethanol has been shown to increase urinary magnesium excretion acutely (83,84); however, this occurs only when the blood alcohol levels are rising. Furthermore, Dunn and Walser (85) showed that

alcohol does not increase urinary magnesium excretion in patients maintained on a low-magnesium diet. Thus, it appears that alcohol-induced renal magnesium wasting is probably not the major mechanism responsible for magnesium depletion. A very important cause for the magnesium depletion in alcoholic patients may be the profound reduction in dietary intake of this cation. In view of the frequency with which hypophosphatemia is associated with hypomagnesemia in alcoholic patients, it seems possible that the magnesium depletion is in part a result of phosphate depletion. A number of investigators have reported increased urinary magnesium excretion in rats (86), dogs (87), and humans (88) during phosphorus depletion, although the mechanism of this phenomenon has not been defined.

Hypomagnesemia also has been observed in patients with hyperthyroidism (89,90). This hypomagnesemic state is associated with an increased exchangeable magnesium pool, suggesting that the thyroid hormone has a direct stimulatory effect on the transport of magnesium into cells. The degree of hypomagnesemia has been correlated with the severity of the hyperthyroid state, with the lowest values found in apathetic thyrotoxicosis (91).

Hypomagnesemia also has been described in patients with severe burns. This probably results from a combination of factors, including lack of oral intake and losses through the denuded skin (92). Following parathyroidectomy, especially in patients with severe bone disease, serum magnesium may fall to subnormal levels (93). The most apparent reason for this reduction in the serum magnesium concentration is the rapid deposition of magnesium in the newly formed bone salts. Finally, hypomagnesemia is not uncommon in patients with uncontrolled diabetes mellitus. It is commonly associated with increased urinary magnesium excretion that is reversed by correction of the hyperglycemia with insulin.

NEONATAL HYPOMAGNESEMIA

Hypomagnesemia can occur in infants as well. Normally, there is a magnesium gradient between the blood of the mother and fetus, with magnesium being slightly higher in fetal blood. However, this gradient is not great enough to protect the fetus if the mother is magnesium depleted (94). Hypomagnesemia has been described in newborns whose mothers have had malabsorption syndromes, have chronically ingested stool softeners, or have had hyperparathyroidism (95). In a series of 20 children with magnesium depletion, the most common cause was the repeated

passage of watery stools regardless of the specific cause (96). A contributory factor was felt to be starvation, which was present in these children. In addition, hypomagnesemia has occurred in association with exchange transfusions, neonatal hepatitis, and polycythemia in infancy (97). Offspring of diabetic mothers also have been reported to have hypomagnesemia (98).

Clinical Consequences of Magnesium Depletion

With the development of atomic absorption spectrophotometry, which made possible the accurate determination of plasma magnesium levels in hospital laboratories, it became apparent that hypomagnesemia is not an uncommon finding, especially in some hospital populations. Patients may have severe hypomagnesemia in the absence of any recognizable symptoms. When symptoms do occur, they are largely confined to the neuromuscular system. These symptoms include weakness, muscle fasciculation, tremors, and positive Chvostek and Trousseau signs (Table 7-5). Generalized tetany occasionally may occur (39,40). The mechanism responsible for the development of tetany is poorly understood, but it is clear that tetany can occur in the absence of either hypocalcemia or alkalosis. A decreased concentration of either magnesium or calcium lowers the threshold to stimulation of a nerve, with resulting increased irritability (99,100). However, their effects are antagonistic in muscle. A low concentration of magnesium enhances muscle contraction, whereas a low concentration of calcium inhibits it (101). Studies suggest that the ECF magnesium depletion may increase acetylcholine action at the nerve ending, which then lowers the threshold of the muscle membrane (39).

Table 7–5 Symptoms of Magnesium Disturbances

Concentration (mEq)	Clinical Manifestation
Hypomagnesemia	
<1.4	Neuromuscular irritability, hypocalcemia, hypokalemia
<0.8	Tetany, seizures, arrhythmias
Hypermagnesemia	
>2.4	Asymptomatic

>4	Lethargy, drowsiness, flushing Nausea and vomiting Diminished deep tendon reflex Somnolence
>6	Loss of deep tendon reflexes Hypotension ECG changes Complete heart block
>10	Cardiac arrest Apnea, paralysis Coma, death

In addition to the symptoms mentioned, patients with magnesium depletion also may have disturbances of the central nervous system, manifested by marked changes in personality, including excessive anxiety and, at times, delirium and frank psychosis (102).

The clinical importance of reduced tissue stores of magnesium is much less clear, although it is well recognized that hypomagnesemia can be associated with clinical symptomatology. Intracellular magnesium depletion with its effect on intracellular potassium may have an adverse effect on myocardial function and its electrophysiologic response.

Biochemical Consequences of Magnesium Depletion

The earliest biochemical alteration during magnesium depletion is a fall in serum magnesium concentration. In growing animals, serum magnesium falls during the first day the animal is on a magnesium-deficient diet (103). Even humans have been shown to have a significant reduction in serum magnesium concentration within 5 to 7 days after being placed on a diet deficient in magnesium (2,6).

Erythrocyte magnesium concentration also has been measured during experimentally induced magnesium depletion in humans and has been found to fall but less rapidly than the plasma magnesium concentration. Because factors other than the status of the body magnesium stores, such as erythrocyte age, also influence erythrocyte magnesium concentration, this determination cannot be used as a valid index of the body magnesium content (104).

A more uniform correlation between total body magnesium and bone

magnesium concentration has been found. In almost every study in which bone magnesium concentration has been measured during magnesium depletion, it has been found to be decreased (7,8). The surface-limited magnesium pool on bone seems to be readily available during magnesium depletion and is rapidly used to replace other body magnesium deficits. In addition, bone magnesium concentration has been shown to strongly correlate with serum magnesium levels in normal, magnesium-deficient, and magnesium-overloaded animals and human subjects (Fig. 7-3) (7,8).

In contrast to bone, there is a poor correlation between plasma magnesium levels and muscle and cardiac magnesium levels (7). However, a reasonably good correlation has been found between mononuclear blood cell magnesium and muscle and heart magnesium (105). Most confusion regarding evaluation of the status of the body magnesium resides around the measurement of muscle magnesium concentrations. Muscle magnesium has been found to be decreased in magnesium-depleted animals (106) but to a lesser extent than bone magnesium. In addition, in a variety of conditions, muscle magnesium has been found to be reduced in association with normal or even increased plasma and bone magnesium levels (7,107,108). There is a close interrelationship between muscle potassium and magnesium levels. During magnesium depletion, in association with the fall in muscle magnesium, there is also a decrease in muscle potassium concentration (7,109).

This change in muscle potassium during magnesium depletion may result from an inability of the muscle to maintain an appropriate gradient for potassium, possibly as a consequence of reduced magnesium-dependent Na^+/K^+ ATPase activity and magnesium's effect on potassium channels (110). A number of investigators have shown that muscle magnesium and potassium concentrations are affected similarly and to a larger extent in primary potassium depletion (7,109) and malnutrition (111), showing that these changes in muscle cation composition are not necessarily characteristic of primary magnesium depletion. It has been found that muscle magnesium falls 0.5 mmol for every 10 mmol fall in muscle potassium during potassium depletion (7). A similar relationship between these two ions exists in the myocardium (112). Because muscle potassium is readily exchangeable and reflects total body potassium, the most likely cause for the reported reduced muscle magnesium levels in patients with a variety of clinical disorders who have normal plasma magnesium levels (107,108) appears to be primary potassium depletion with secondary muscle magnesium depletion.

Therefore, bone and ECF magnesium are the available magnesium

pools used to replenish soft-tissue magnesium deficits during magnesium depletion. Furthermore, serum magnesium concentration, not muscle, best reflects bone magnesium content and can be used as an indicator of total body magnesium stores.

Besides the measurement of magnesium concentration in biologic tissues and fluids, the retention of magnesium following an acute intravenous infusion of magnesium also has been used to estimate the status of the body magnesium stores. Normal individuals in magnesium balance excrete the majority of a systemically administered magnesium load in 24 to 48 hours. In contrast, individuals with magnesium deficits retain a significant fraction of the injected magnesium (105,113).

Effect of Magnesium on Calcium Metabolism

Severe magnesium depletion has been found to alter calcium metabolism significantly in animals as well as humans. Studies in cattle (114), sheep (115), pigs (116), dogs (117), monkeys (118), rats (119), and humans (40) have shown that severe magnesium depletion is associated with hypocalcemia in all these species. Subsequently, calcium balance studies in animals, as well as humans, have shown that with the development of hypocalcemia during magnesium depletion, external calcium balances remain unchanged or actually become more positive. Thus, hypocalcemia results from alterations in internal control mechanisms for calcium. The interrelationship between magnesium and PTH is quite complex. Acutely, magnesium appears to have a direct effect on PTH secretion (120). Perfusion studies of goat and sheep parathyroid glands have shown that low magnesium concentration acutely stimulates the release of PTH. In vitro studies (121) showed a first-order relationship between PTH release and the combined concentrations of calcium and magnesium. That these divalent cations had an equivalent effect on hormone release was shown by the finding that PTH secretion was unchanged when either cation was decreased in association with a corresponding increase of the other cation.

Parathyroid function appears to be affected in an opposite direction during chronic magnesium depletion. Immunoreactive PTH levels usually have been found to be normal, which is inappropriately low for the hypocalcemic and hypomagnesemic state, or actually low (122–124). Recent studies provide support for an abnormality in PTH release in chronic magnesium depletion. Anast et al. (125) found that PTH levels increased within 5 minutes of administration of magnesium intravenously.

Mennes et al. (126) also found that PTH levels rapidly increased in hypomagnesemic patients following intravenous administration of magnesium. Thus, it appears that chronic magnesium depletion is associated with suppression of PTH release from the parathyroid glands rather than a direct effect on decreasing synthesis of this hormone. In further support of this is a recent finding of suppression of PTH secretion by magnesium depletion in a patient with pseudohypoparathyroidism, with subsequent elevated PTH levels noted following magnesium repletion (127). It is interesting that, although chronic magnesium depletion suppresses PTH release, it has little effect on other endocrine glands. Cohan et al. (128) showed normal responsiveness of the adrenal cortex, thyroid, gonads, and liver to their respective trophic hormones in hypomagnesemic patients.

A number of studies have suggested that hypomagnesemia-induced hypocalcemia also results from skeletal resistance to PTH. In vitro techniques (129) revealed that PTH caused less calcium release from fetal rat bone when the medium was low in magnesium. Similarly, bones from magnesium-depleted animals were found to release less calcium and cAMP when exposed to PTH than control bones (130).

Data obtained from in vivo studies have yielded conflicting results. Studies in magnesium-deficient dogs (131), rats (132), and monkeys (118) have shown a normal calcemic response to PTH. In contrast, PTH resistance was found in magnesium-deficient chicks as assessed by the hypercalcemic response (133,134).

Studies in humans also have yielded conflicting results. The earliest studies performed by Estep et al. (135) in hypomagnesemic alcoholic patients, Muldowney et al. (136) in patients with hypomagnesemia secondary to malabsorption syndromes, and Woodard et al. (137) in patients with diarrhea showed an impaired calcemic response from PTH. However, Chase and Slatopolsky (122) found a normal calcemic response from PTH in two hypocalcemic hypomagnesemic adults. A normal calcemic response from PTH has been found in the majority of hypomagnesemic children studied (138).

In human studies (139) as well as some animal studies (140), $1,25(OH)_2D_3$ levels have been found to be low during magnesium-induced hypocalcemia. However, the hypocalcemia does not appear to be related to the low $1,25(OH)_2D_3$ levels, because the hypocalcemia responds to magnesium replacement and the replacement has no effect on vitamin D levels.

Therefore, it can be concluded that several factors may be involved in

the pathogenesis of magnesium depletion-induced hypocalcemia. Abnormal PTH release in response to a hypocalcemic stimulus has been well established. There appears to be altered bone solubility as well, possibly as a result of loss of magnesium ions from the crystal surface and hydration shell, with replacement by calcium ions by heteroionic exchange. This could render the bone resistant to PTH as well as other factors that tend to solubilize bone salts. It is possible that PTH secretion is impaired early in the course of magnesium depletion, whereas there is a combination of suppression of PTH release and bone resistance to PTH later in the course of magnesium depletion. This might explain the discrepancies noted in the preceding studies, with the difference in bone response to PTH related to the duration and magnitude of magnesium depletion.

Effect of Magnesium Depletion on Potassium and Other Intracellular Constituents

Whang et al. (141), in studying 106 patients with hypokalemia, found that 42% were also hypomagnesemic. Similarly, Boyd et al. (142) reported a 38% incidence of coexisting hypomagnesemia in hypokalemic patients. However, Watson and O'Kell (143) found a much lower incidence, with only 7.4% of 136 hypokalemic patients also hypomagnesemic. This difference can possibly be explained by the type of patient population studied. The patients studied by Whang et al. (141) were from a University and Veterans Administration hospital, whereas Watson and O'Kell (143) studied patients hospitalized at a tertiary community hospital. From these studies, it would appear that in certain patient populations, such as alcoholic and diabetic patients with ketoacidosis, the combined disturbance of hypokalemia and hypomagnesemia may occur quite commonly.

Whang et al. (144) have suggested that two types of potassium depletion coexist with magnesium depletion. The first represents a combination of intracellular and extracellular potassium and magnesium depletion, whereas the second type represents only intracellular depletion of these two cations. Irrespective of the type of depletion, repletion of potassium frequently cannot be accomplished without the concomitant administration of magnesium. Whang et al. (144) and Rodriguez et al. (145) have used the term "refractory potassium repletion states" to

describe this condition. The most common cause has been the use of diuretics to treat edematous disorders. In a review by Whang et al. (144), diuretic therapy was responsible for 63% (46 of 73) of the patients reported who had potassium depletion refractory to only potassium replacement. In the remaining patients, it resulted from a variety of disorders including Bartter syndrome, familial hypokalemic alkalosis, burns, and alcoholism. Whang et al. (144) and Dyckner and Webster (146) have also found that potassium replacement alone may not increase muscle potassium and that a combination of potassium and magnesium replacement is required to normalize muscle potassium and magnesium. Animal studies would add additional support to this contention. Whang and Welt (147) showed that potassium losses from the rat diaphragm maintained in a low-magnesium bath could be prevented by adding more magnesium to the bath. Studies using the isolated rat interventricular septa have shown that increasing extracellular magnesium abruptly decreases ^{42}K efflux (148). Magnesium also has been shown to reduce or prevent the net potassium loss from the heart induced by glycosides (149). This was further supported by showing that a magnesium infusion in animals receiving acetylstrophanthidin prevented potassium loss from the myocardium as determined by measuring arterial and coronary sinus potassium concentrations (150). The findings suggest that this effect of magnesium on intracellular potassium is a result of magnesium enhancing Na^+/K^+ ATPase activity (151). However, this has been criticized by Shine (149), who suggests as an alternative that there might be a direct effect of magnesium on potassium channels in the sarcolemmal membrane or that magnesium's effect might be one of competing with calcium for cellular uptake. An additional factor that may be involved in magnesium-induced potassium depletion is aldosterone. During experimental magnesium depletion-induced kaliuresis, aldosterone levels have been found to be increased, and the kaliuresis can be abolished with spironolactone (152). However, somewhat at variance with this is the finding that magnesium infusion decreases urinary potassium excretion in patients with Bartter syndrome without affecting plasma renin and aldosterone levels (153). This suggests that magnesium repletion may modify kaliuresis by means other than or in addition to its effect on the renin–aldosterone system.

Although most investigators have felt the intracellular alteration in potassium and magnesium to be a result of magnesium depletion, it seems equally as likely, if not more likely under some conditions, that the disturbances are a consequence of primary potassium depletion with secondary magnesium depletion. Studies have shown that potassium can

affect the cellular concentration of magnesium. House and Bird (154) showed that goats placed on a high-potassium diet retained more magnesium than goats maintained on a normal potassium intake, given a similar intravenous magnesium load. In addition, as stated earlier, it has been well documented that in primary potassium depletion, intracellular magnesium is also reduced (7,109). This interrelationship between magnesium and potassium could have considerable clinical importance. Under a variety of conditions, it is impossible to replace intracellular deficits of magnesium and potassium without giving both of these cations together, whether the deficiency resulted from either primary potassium or magnesium depletion. In addition, a small number of patients have been reported who have developed tetany following potassium supplementation (155). Although magnesium levels have not been measured in all of these patients, it seems likely in view of their underlying diseases that magnesium deficiency was also present.

Further support for the relationship between these two cations comes from the finding that increased extracellular magnesium causes an abrupt decrease in potassium efflux from the rat intraventricular septa (148). Magnesium also decreased glycoside-induced potassium loss from the myocardium (150). One mechanism by which magnesium may enhance intracellular potassium is by stimulating Na^+/K^+ATPase activity, thus allowing the cell to maintain a potassium gradient (151).

Besides its effect on intracellular potassium, magnesium depletion also causes intracellular phosphorus depletion in muscles. Cronin et al. (156) described rhabdomyolysis in magnesium-depleted dogs and suggested that this resulted from magnesium depletion-induced intracellular phosphate depletion.

Effect of Magnesium on Cardiovascular Function

The effect of magnesium on cardiovascular function has received increasing attention during the last decade. Extracellular and/or intracellular magnesium depletion has been implicated in a variety of cardiovascular disturbances, including ventricular arrhythmias, digitalis intoxication, modulation of vascular tone, and atherogenesis.

Cardiac arrhythmias are an important complication of magnesium depletion, especially in patients on digitalis. Magnesium depletion has been associated with a prolonged QTc interval (157). In addition,

magnesium supplementation has been shown to reduce the QTc intervals even in patients with normal serum magnesium levels (158,159). Torsades de pointes is a repetitive polymorphous ventricular tachycardia that occurs in the presence of QT prolongation, which is usually induced by drugs that prolong the QT interval. Because of its effect on prolonging the QT interval, magnesium depletion also has been implicated in the pathogenesis of torsades de pointes, although such a relation has rarely been shown (160). Because of its ability to shorten the QT interval, magnesium supplementation has been used with some success in treating torsades de pointes (161,162).

With regard to cardiac function, there is a close association between magnesium and potassium. Magnesium has been shown to attenuate the electrophysiologic effects of hyperkalemia (163). Furthermore, in view of the relationship between magnesium and intracellular and extracellular potassium depletion, magnesium depletion has been implicated as a potential cause of digitalis intoxication (164). This is supported by the finding that an acute induction of hypomagnesemia in dogs with dialysis facilitates the development of digitalis intoxication and arrhythmias (151). Moreover, ventricular arrhythmias, including those induced by digitalis, are sensitive to magnesium therapy (165). However, it is unclear how much of these cardiovascular alterations are a direct result of magnesium depletion or else a consequence of the associated intracellular potassium depletion.

Therapy of Magnesium Deficiency

Magnesium replacement is indicated in all patients with hypomagnesemia whether symptomatic or not. Symptoms are unusual unless serum magnesium levels are <0.8 mEq/L. Adequate replacement of magnesium deficits usually can be accomplished through dietary sources alone in patients with mild asymptomatic hypomagnesemia. Patients with severe symptomatic hypomagnesemia usually require parenteral replacement of magnesium deficits. Magnesium may be useful, at least as adjuvant therapy, in treating a variety of tachyarrhythmias, including torsades de pointes and some associated with digitalis toxicity. As stated, uncorrected intracellular magnesium deficiency can impair repletion of cellular potassium. At this time, it cannot be recommended that all patients with potassium depletion be given both potassium and magnesium replacement. However, patients with potassium depletion in association with any

documented amount of hypomagnesemia should receive combined replacement with both of these cations. In addition, magnesium supplementation should be strongly considered in patients with severe potassium depletion or in those who appear to be resistant to potassium replacement.

Because the kidneys have a marked capability for excretion of magnesium, excessive magnesium treatment usually results in only temporary hypermagnesemia. However, when a patient has compromised kidney function, magnesium should be administered cautiously and with close monitoring of the plasma magnesium levels. The different compounds commonly used for magnesium replacement, including their molecular weights and percent magnesium by weight, are listed in Table 7-6.

Table 7–6 Magnesium Salts Used for Replacement or Phosphorus Binder

Compound	Molecular Weight	% Mg by Weight
$MgCl_2\ 6H_2O$[a]	203.23	11.96
$MgSO_4\ 7H_2O$[b]	246.50	9.86
$Mg(C_2H_3O_2)_2\ 4H_2O$[c]	214.47	11.33
$Mg(OH)_2$[d]	58.3	41.68
MgO[e]	40	60.30
$MgCO_3$[f]	84.32	28.82

[a]Magnesium chloride.
[b]Magnesium sulfate.
[c]Magnesium acetate tetrahydrate.
[d]Magnesium hydroxide.
[e]Magnesium oxide.
[f]Magnesium carbonate.

Magnesium deficit can be roughly calculated by assuming that the space of distribution is slightly larger than the ECF volume. This assumption seems to be valid because during magnesium depletion, soft-

tissue magnesium stores are affected little, if at all, and only the surface-limited pool of magnesium on bone would equilibrate during repletion. Therefore, it appears that replacement therapy may be adequate for a number of hypomagnesemic patients if only 30% as much magnesium as recommended by Flink (166) is used. However, in some conditions, the magnesium deficit may be in excess of this amount. It has been estimated that patients with diabetic ketoacidosis may retain 40 to 80 mEq (480–960 mg) of magnesium over 2 to 6 days following recovery (76,167). In some alcoholic patients, deficits of up to 1 mEq/kg have been found (168).

Whenever possible, intravenous magnesium replacement should be avoided in small children because of the danger of hypotension. For children weighing 4 to 7 kg, a safe initial dose is 0.5 mL of 50% $MgSO_4$ (2 mEq of magnesium) given intramuscularly. For heavier children, 1.0 mL of 50% $MgSO_4$ may be given intramuscularly (169).

Table 7–7 Magnesium Replacement

Intravenous Administration (50% $MgSO_4$)
Symptomatic emergent (seizures) (50% $MgSO_4$) 4 mL (16.3 mEq, 8.2 mmol, or 195 mg of Mg) diluted to 100 mL infused over a 10-min period
Symptomatic Nonemergent
Day 1: 12 mL (49 mEq of Mg) in a 1,000-mL solution containing glucose infused over 3 h, followed by 10 mL (40 mEq of Mg) in each of two 1-L solutions administered throughout the day Days 2–5: 12 mL (49 mEq of Mg) distributed equally in total daily IV fluids
Oral Therapy (MgO)
250–500 mg (12.5–25 mEq of Mg) t.i.d. to q.i.d.
Intramuscular Route (50% $MgSO_4$) (Painful)
Day 1: 4 mL (16.3 mEq of Mg) q2h for 3 doses and then q4h for 4 doses Day 2: 2 mL (8.1 mEq of Mg) q4h for 6 doses Days 3–5: 2 mL (8.1 mEq of Mg) q6h

For adults with normal kidney function, the suggested magnesium replacement is given in Table 7-7 (166,170). Oral replacement therapy is

limited by the amount of magnesium administration that causes diarrhea. Oral replacement also can be made with antacids that contain both magnesium and aluminum salts or magnesium calcium salts in patients who develop diarrhea from magnesium oxide replacement. Patients maintained only on intravenous therapy for periods in excess of 5 to 7 days may require some magnesium supplementation to prevent the development of magnesium depletion. This can be accomplished by giving 100 mg (8 mEq) of magnesium daily if there are not excessive losses of this cation through the kidneys and GI tract. For management of arrhythmias, it has been suggested that 8 mmol of magnesium sulfate be administered intravenously over 1 minute or that as much as 1.5 mmol/kg be given over a 10-minute period (155,164,165,171,172).

Hypermagnesemia

Most cases of hypermagnesemia are seen in patients with CKD but can be seen with shifts or exogenous administration. Normally, plasma magnesium concentration increases in the hibernating animal (173) and during hypothermia (174). Magnesium is commonly given parenterally for the treatment of eclampsia. Blood levels of magnesium usually are increased to 6 to 8 mEq/L but occasionally may be as high as 14 mEq/L (175). This may result in neonatal hypermagnesemia, but in general, blood levels tend to be lower in the infant than in the mother. Other states in which hypermagnesemia has been described with some frequency are in patients with kidney failure (176) and adrenocortical insufficiency (177). The majority of patients with far advanced kidney failure have a modest elevation of serum magnesium levels (178). Severe hypermagnesemia occurs most frequently in patients with marked kidney disease who are given large amounts of oral magnesium salts, usually in the form of magnesium-containing antacids (179,180). Although the normal kidney has a great ability to excrete magnesium, magnesium intoxication can occur in patients with normal kidney function (181,182). This usually results from an individual inadvertently receiving a large oral load of hypertonic magnesium salts. This results in two phenomena that can cause life-threatening hypermagnesemia. First, excessive magnesium is absorbed. Second, and possibly of greater importance, the hypertonic solution pulls fluid from the extracellular space into the GI tract, which causes volume depletion and decreases kidney function, which in turn compromises the excretion of the absorbed magnesium. Hypermagnesemia

is being seen with increasing frequency in patients with drug overdoses because of the magnesium-containing laxatives commonly used to treat this condition (183). Elderly patients with GI disorders receiving magnesium-containing compounds are at risk of developing hypermagnesemia (184).

Symptoms of Acute Magnesium Intoxication

Acute elevations of the serum magnesium levels depress the central nervous system as well as the peripheral neuromuscular junction (Table 7-5). Magnesium in pharmacologic doses has a curare-like action on neuromuscular function. This is probably caused by inhibition of the prejunctional release of acetylcholine owing to displacement of membrane-bound calcium at the neuromuscular junction, which then decreases the depolarizing action of acetylcholine (185). Magnesium also increases the stimulus threshold in nerve fibers, and direct application of magnesium to the central nervous system blocks synaptic transmission. Electrophysiologic studies demonstrate reduced compound muscle action potential amplitudes, decremental amplitude responses to repetitive stimulation at low rates, and a marked amplitude increment following brief exercise (186).

The deep tendon reflexes are depressed when serum magnesium levels exceed 4 mEq/L. A flaccid quadriplegia may develop in the patient at magnesium levels >8 to 10 mEq/L. Deep tendon reflexes are absent in this stage. The patient is typically conscious and reasonably alert. However, because of marked muscle weakness, there is difficulty talking and swallowing, and respiratory paralysis is a real hazard (102). Other symptoms include lethargy, nausea, dilated pupils, and respiratory depression (179,180). There also may be smooth muscle paralysis, resulting in difficulty in micturition and defecation (180). Hypotension and bradycardia are common, and in rare cases, cardiac arrhythmias consisting of complete heart block and cardiac arrest have been observed (180).

Treatment of Acute Magnesium Intoxication

Treatment of hypermagnesemia is primarily directed at reducing the serum magnesium levels. However, calcium acts as a direct antagonist to

magnesium, and the injection of as little as 5 to 10 mEq of calcium may readily reverse a potentially lethal respiratory depression or cardiac arrhythmia (102). Thus, intravenous calcium should be used as the initial treatment modality when life-threatening complications of magnesium intoxication are present.

Any parenteral or oral magnesium salt the patient has been taking should be discontinued immediately. Intravenous furosemide should be administered and urine volume replaced with 0.5 N saline if kidney function is adequate. This approach ensures continuing urine output and prevents volume depletion. Calcium gluconate (15 mg of calcium/kg), given over a 4-hour period, also can be used to increase urinary calcium excretion and, in turn, magnesium excretion. In patients with severe impairment of kidney function, dialysis may be required. This should be carried out with a dialysate free of magnesium. The serum magnesium usually can be decreased to a safe level in 4 to 6 hours of dialysis (180).

Chronic Magnesium Excess

CKD is the only state thus far described in which there can be a chronic excess of total body magnesium (181–186). The body magnesium pools increased during magnesium excess are ECF magnesium and bone magnesium (176). Decreases in the dialysate magnesium concentration since the early days of renal replacement therapy have raised new questions about magnesium balance in dialysis patients. No quantitative bone analyses are available to evaluate the net effect on magnesium balance since these early studies of Alfrey (176). Magnesium has been shown to be an integral part of the soft-tissue calcium phosphate deposits found in uremic patients (187) and in vascular calcification in animal models (188), suggesting that this cation might in part be responsible for this complication. However, in vitro, magnesium impairs hydroxyapatite crystal growth (189,190) and a few observational studies found less vascular and valvular calcification in dialysis patients with higher serum magnesium concentrations (191,192). Furthermore, magnesium has been shown to be an effective phosphate binder (193,194) and has been used in earlier times to decrease aluminum exposure (193) and more recently to decrease net calcium intake (195,196). Additional studies are necessary to determine magnesium balance in dialysis patients and to investigate whether modifications in serum levels or magnesium balance have adverse or favorable consequences with regard to bone disease, vascular

calcification, and cardiovascular outcomes.

Summary

The chemistry of magnesium is unique among cations of biological relevance. Magnesium has the atomic number 12 and is classified as an alkaline earth element (group 2) within the periodic table of element (197). Subsequently recognized as an element and isolated by Humphrey Davy from magnesia (31), the role of magnesium in the human body emerged once magnesium was determined in blood plasma in 1920 (32). The improved procedures for magnesium measurement have furthered understanding of the role of magnesium in health and disease. Magnesium measurement has now become a routine procedure in most laboratories. It has become apparent that disorders of magnesium metabolism occur with a frequency almost as great as those noted for other major body elements. Genetic screenings and microarray-based expression studies have resulted in the identification of numerous magnesium-transporting proteins. The understanding of genetic and drug-induced disorder of magnesium homeostasis has enhanced our knowledge of normal and abnormal magnesium metabolism.

Hypomagnesemia is found with some frequency in a variety of conditions, including small bowel disease, chronic alcoholism, malnutrition, endocrine abnormalities, posttransplant patients, and in certain kidney diseases. At times, the severity of magnesium depletion may be so great that such symptoms as tetany, delirium, psychosis, or even convulsions may occur. The route of magnesium repletion varies with the severity of clinical manifestations. Patients with severe signs and symptoms of hypomagnesemia should receive intravenous magnesium with cardiac monitoring. Oral replacement should be given to asymptomatic patients. A typical daily dose in a patient with normal renal function is 240 to 1,000 mg (20–80 mEq) of elemental magnesium in divided doses. The underlying diseases should also be corrected. Patients with hypomagnesemia induced by a thiazide or loop diuretic that cannot be discontinued may benefit from the addition of a potassium-sparing diuretic such as amiloride. It may also be useful in conditions associated with persistent urinary magnesium wasting such as Gitelman or Bartter syndrome. Great caution should be exercised in treating patients who have acute or chronic kidney injury with magnesium-containing medications. Patients with reduced kidney function may require magnesium repletion

only if they have severe hypoglycemia.

Although acute magnesium intoxication has been repeatedly observed and can lead to death as a result of arrhythmias and respiratory arrest, the consequences of chronic magnesium excess have not been well defined and deserve further study. At magnesium concentrations up to 1.5 mmol/L (3.6 mg/dL), hypermagnesemia is asymptomatic. Treatment consists of cessation of magnesium administration and intravenous infusion of calcium.

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CHAPTER 8

Disorders of the Renin–Angiotensin–Aldosterone System

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The renin–angiotensin–aldosterone system (RAAS) has been conserved through evolution and is present in all vertebrates (1). It has long been known to play a central role in the regulation of blood pressure and renal sodium and water excretion. The classic, linear description of the RAAS begins with the conversion of the liver-derived glycoprotein angiotensinogen into the inactive angiotensin (Ang) I by renin, a protease secreted by the juxtaglomerular (JG) cells of the kidney. Ang I is then cleaved by angiotensin-converting enzyme (ACE) into Ang II, which has a diverse range of physiologic effects, including acting as an aldosterone secretagogue in the adrenal cortex. Aldosterone stimulates sodium reabsorption in the distal nephron. The system forms a feedback loop whereby secretion of the main effectors is affected by other members of the RAAS cascade and by other mediators.

The understanding of this system has become increasingly complex over the past two decades with the identification of new Ang receptors, a (pro)renin receptor and additional Ang peptides (Fig. 8-1). Numerous disparate physiologic and pathophysiologic effects have been attributed to the angiotensins and to aldosterone. The notion of the RAAS as an

endocrine system has been expanded to include paracrine and autocrine effects with local production of Ang II at the tissue level. This chapter begins with an overview of each component of the RAAS, followed by a discussion of the pathophysiologic importance of each. We then discuss RAAS blockade and its importance in the treatment of individuals with chronic kidney disease (CKD).

Angiotensinogen

Angiotensinogen is a 55- to 60-kDa serum glycoprotein that serves as the precursor for all Ang peptides. It is the sole circulating renin substrate; cleavage of a leucine–valine bond produces the decapeptide Ang I. The variability in molecular size of angiotensinogen is attributed to different patterns of glycosylation, as the angiotensinogen gene encodes only a single protein product (2). A high-molecular-weight variant has also been identified that is normally present at low levels. It exists as a greater fraction of total plasma angiotensinogen during the third trimester of pregnancy and is associated with hypertension and preeclampsia in pregnant women (3).

Angiotensinogen is primarily synthesized and released by the liver. Hepatic production may be increased by a number of factors. Angiotensinogen is an acute-phase reactant, and its synthesis is stimulated by stressors such as infection. Glucocorticoids, estrogens, and thyroxine increase angiotensinogen production by the liver (4). Ang II stimulates angiotensinogen synthesis in a positive feedback loop, leading to greater presence of the proximal substrate of the renin–angiotensin system (RAS) at times of increased Ang II production (5).

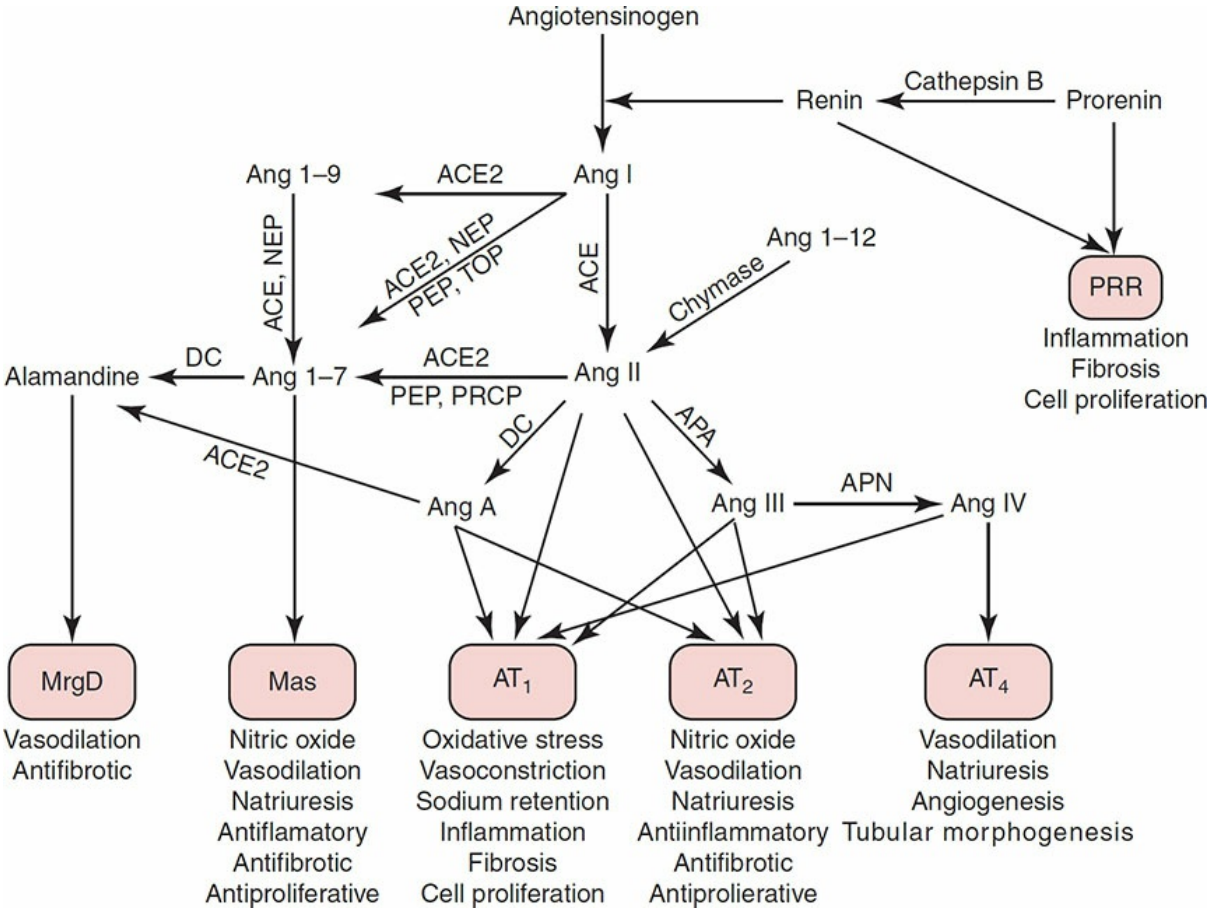

Figure 8–1 Current view of the renin–angiotensin system cascade including the renal effects of receptor stimulation. ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; APA, aminopeptidase A; APN, aminopeptidase N; AT_1, angiotensin I receptor; AT_2, angiotensin II receptor; AT_4, angiotensin IV receptor; DC, decarboxylase; Mas, angiotensin 1–7 receptor; NEP, neutral endopeptidase; MrgD, Mas-related G protein-coupled receptor D; PEP, prolyl endopeptidase; PRCP, prolyl carboxypeptidase; PRR, (Pro)renin- receptor; TOP, thimet oligopeptidase.

There is evidence of local angiotensinogen production in multiple organ systems as well. Angiotensinogen mRNA expression has been demonstrated in the kidney, heart, vascular tissues, adrenal gland, central nervous system, fat, and leukocytes (2,6). Within the kidney, angiotensinogen mRNA is most abundant in the cortex, especially in the proximal tubule, but it is also present in the glomerulus and medulla (7).

Renin

Renin is a 37- to 40-kDa aspartyl protease with high specificity for angiotensinogen, its only known substrate. Translation of renin mRNA

yields the inactive precursor preprorenin, which is converted to prorenin by the removal of a 23-amino acid signal peptide from the carboxyl terminus during insertion into the endoplasmic reticulum (8). Prorenin is a proenzyme that may be rapidly and directly secreted in the intact form or packaged into immature granules and processed into the active renin. Both prorenin and renin are secreted by the JG cells of the kidney but the former is the major circulating form as its plasma concentration is 10-fold higher than that of renin (9). Some have speculated that prorenin may be converted to renin in the circulation or locally in tissues, and prorenin-activating enzymes have been found in vascular endothelial cells and neutrophils (10). Renin mRNA has also been demonstrated in multiple organs other than the kidney, including the brain, liver, lung, submandibular gland, prostate, testis, ovary, spleen, pituitary, and thymus (11). Nevertheless, extrarenal production of renin has not been clearly demonstrated, and extrarenal sites that express the renin gene secrete prorenin, not renin (12).

A functional renin receptor, called the (pro)renin receptor, possesses both renin- and prorenin-specific binding. It has been localized to the mesangium in glomeruli, smooth muscle cells in renal and coronary arteries, placenta, brain, and liver (13). Binding of renin and prorenin increases the catalytic efficiency of angiotensinogen cleavage and also induces intracellular signaling via activation of the mitogen-activated protein (MAP) kinases ERK1 and ERK2 (13). Through activation of this pathway, (pro)renin receptor binding increases the expression of profibrotic molecules including transforming growth factor (TGF)-β1, plasminogen activator inhibitor-1 (PAI-1), fibronectin, and collagen I (14–16), as well as the proinflammatory mediators interleukin-1β (IL-1β), cyclooxygenase-2, and tumor necrosis factor-α (TNF-α) (17,18). These studies suggest a functional role for prorenin via nonproteolytic activation induced by receptor binding. They also demonstrate the Ang II-independent, receptor-mediated effects of renin. As such, renin itself may contribute to the progression of CKD by promoting fibrosis and inflammation.

Regulation of Renin Secretion

The majority of renin production occurs in the JG cells on the afferent arterioles of the kidney. In normal subjects, sodium intake is the main determinant of renin secretion. Low sodium intake, resulting in reduced

extracellular volume, stimulates renin release. Conversely, high sodium intake inhibits renin secretion through extracellular volume expansion. Several mechanisms, which primarily sense volume changes, regulate renin production and secretion (Table 8-1).

RENAL BARORECEPTORS

The JG cells, of myoepithelioid origin, sense changes in renal perfusion pressure through changes in stretch of the afferent arteriolar wall. JG cells increase renin secretion in response to decreased stretch with reduced perfusion pressure. Conversely, renin secretion is inhibited in response to increased pressure or stretch within the afferent arteriole. The coupling of perfusion pressure with renin release is likely mediated by changes in cytosolic calcium concentration related to JG cell stretch (19).

MACULA DENSA

Renin secretion is also regulated by the macula densa, an area of closely packed specialized tubular cells in the thick ascending limb of the loop of Henle. The composition of tubule fluid delivered to the macula densa regulates renin release via an ion-sensing mechanism that is independent of volume. Infusion of sodium chloride produces a rapid decline in plasma renin activity (PRA) that is not seen with comparable volume expansion with a dextran-containing solution (20). While this effect was initially thought to be sodium dependent, further studies demonstrated the importance of the chloride concentration of tubular fluid. PRA is not suppressed by infusion of non–chloride-containing sodium solutions, but it is suppressed by non–sodium-containing chloride solutions (21). Thus, renin inhibition is thought to be related to the magnitude of chloride absorption in the macula densa. Isolated perfusion of these structures has shown that lower sodium chloride in the lumen of the macula densa stimulates renin secretion. This depends on salt entry into macula densa cells via the $Na^+/K^+/2Cl^-$ cotransporter (22). As the tubular cells of the macula densa are not in direct contact with the afferent arteriolar JG cells, additional mechanisms involving second messenger signaling and paracrine factors have been postulated. Adenosine inhibits renin secretion and has been proposed as a mediator of macula densa-regulated renin release (23).

Table 8–1 Factors Regulating Renin Secretion

Major Stimuli	Major Inhibitors
Decreased renal perfusion pressure	Increased renal perfusion pressure
Decreased NaCl delivery to the macula densa	Increased NaCl delivery to the macula densa
β-adrenergic stimulation	Angiotensin II
Other Stimuli	**Other Inhibitors**
Prostaglandins (PGE_2, PGI_2)	Adenosine
Dopamine	Atrial natriuretic peptide
Glucagon	Endothelin
Nitric oxide	Vasopressin
—	Calcium
—	Vitamin D

NEURAL MECHANISMS

Neural mechanisms modulate renin release, primarily via the sympathetic nervous system. This appears to be mediated by β-adrenergic receptors, based on several lines of evidence. The JG apparatus (JGA) is densely innervated with sympathetic nerves, and β_1-adrenoreceptors have been localized to the JGA and glomerulus (24–26). β-adrenergic agonists and increased renal sympathetic nerve activity stimulate renin release, while β-adrenergic antagonists inhibit this effect and reduce renin secretion (23). The neural modulation of renin release appears to involve the adenylyl cyclase signaling pathway via cyclic adenosine monophosphate (cAMP).

ENDOCRINE AND PARACRINE MECHANISMS

Multiple circulating factors regulate the release of renin. The most important of these is Ang II, which affects renin release through a negative feedback loop that is independent of volume or tubular transport processes. Higher Ang II levels directly inhibit renin secretion by regulating renin gene expression in the afferent arteriole (27,28). ACE inhibition increases renal renin mRNA expression in animal models (27,29) and increases PRA in humans, at least in part, by interrupting the inhibitory feedback of Ang II (30).

Numerous other hormonal influences affect renin levels via both endocrine and paracrine mechanisms (12). Activators of adenylyl cyclase, by increasing cAMP levels, stimulate renin secretion. These include prostaglandin E_2, prostacyclin, dopamine, and glucagon. Nitric oxide indirectly increases cAMP levels and thus also promotes renin release. Natriuretic hormones, such as atrial natriuretic peptide (ANP), inhibit renin secretion via guanylyl cyclase activation. Calcium has been known to suppress renin release from JG cells. This effect is mediated by calcium-dependent inhibition of adenylyl cyclase (31). As such, calcium-liberating hormones such as endothelin, vasopressin, and adenosine block renin secretion as well. Vitamin D appears to negatively regulate renin expression via a calcium-independent mechanism (32).

Angiotensin-Converting Enzyme

ACE is a zinc-containing metalloprotease with a molecular weight of approximately 200 kDa. It is a type I ectoprotein with a long ectodomain that includes the enzymatic active site, a transmembrane domain, and a short cytoplasmic domain (33). ACE cleaves the two C-terminal amino acids (His-Leu) from Ang I to form the octapeptide Ang II. The conversion of Ang I to Ang II occurs rapidly throughout the vasculature. ACE is located on the surface of endothelial cells in many vascular beds, including the kidney. Because the pulmonary vasculature is the main site of ACE synthesis, a single pass through the lung produces nearly complete conversion of Ang I to Ang II (34).

In contrast to the substrate specificity of renin, multiple small peptides are hydrolyzed by ACE, including bradykinin, enkephalins, substance P, and luteinizing hormone-releasing hormone (35–39). Thus, in addition to generating the potent vasoconstrictor Ang II, ACE degrades the vasodilator bradykinin into inactive fragments. This underscores the multiple biologic pathways in which ACE may play an important role. The plasma concentration of ACE may be affected by a number of disease states, including hypothyroidism, diabetes mellitus, sarcoidosis, and other granulomatous diseases (40–42). Yet despite significant variation in ACE levels even among healthy subjects, a significant association between levels of the enzyme and the risk of hypertension has not been identified (43,44).

The human ACE gene has been localized to chromosome 17 (43).

There are two isoforms of ACE, a larger somatic ACE (sACE) and a smaller germinal ACE (gACE). gACE is expressed in sperm cells, likely regulated by androgens, and plays a role in male fertility (45). A soluble form of ACE, formed by cleavage of the ectodomain of sACE, exists in plasma. While its function is not fully understood, soluble ACE is enzymatically active and appears to play a role in renal development and function, but not blood pressure (46).

Within the kidney, ACE is expressed in the brush border of the proximal tubule and on vascular endothelial cells (47,48). Postulated functions for ACE located on the brush border membrane of the proximal tubule include cleavage of filtered peptides for subsequent uptake by epithelial cells and local production of Ang II within proximal tubule fluid to facilitate reabsorption (48).

ACE expression in the vasculature is not limited to the endothelium. Macrophages and other inflammatory cells are significant sources of tissue ACE in human atherosclerotic plaques (49). An identified insertion/deletion (I/D) polymorphism of a 287-base-pair DNA fragment within the ACE gene is associated with clinical outcomes. Specifically, the deletion polymorphism is associated with higher ACE levels (44) and with increased risk of microalbuminuria, retinopathy, and left ventricular hypertrophy in hypertensive subjects (50). The D allele is also associated with kidney disease in individuals with hypertension (51) and diabetes (52). Patients with diabetic nephropathy homozygous for the D allele compared to homozygosity for the I allele may benefit less from the renoprotective effects of ACE inhibitors (53).

Angiotensins

Ang II is a highly potent vasoconstrictor with multiple actions discussed in more detail later. Ang II is formed from cleavage of the C-terminal dipeptide of Ang I by ACE. Alternative pathways for Ang II generation have been demonstrated, but their physiologic importance is unclear. Tonins, cathepsins, and kallikreins can form Ang I or Ang II directly from angiotensinogen (54). Recent evidence suggests that chymase-dependent pathways may be important for Ang II generation within cardiovascular tissues (55). The half-life of Ang II in the circulation is approximately 1 to 2 minutes. It is hydrolyzed by aminopeptidase A to form Ang III, the heptapeptide Ang 2-8 (56). Ang II is also converted to Ang 1-7 and Ang A.

Ang III is a less potent peripheral vasoconstrictor than Ang II (57,58), with a much shorter plasma half-life (59). This is likely due to its greater affinity for angiotensin type 1 (AT_1) receptors than angiotensin type 2 (AT_2) receptors. There is no known receptor specific to Ang III. In AT_1 receptor-blocked rats, Ang III produces natriuresis and vasodilation (60). In the 1970s, Ang III was shown to stimulate aldosterone release from the adrenal zona glomerulosa (61). It also appears to be an important mediator of the RAS in the central nervous system. Intracerebroventricular injection of Ang II and Ang III produces equivalent pressor and drinking responses (58,62). Ang III may be the main effector of vasopressin release in the brain (63). Further cleavage by aminopeptidase N converts Ang III to Ang IV, the hexapeptide Ang 3-8. The role of Ang IV on renal blood flow is somewhat controversial. Initial studies demonstrated that, in anesthetized rats, intrarenal infusion of Ang IV increased natriuresis and renal cortical blood flow, the effect of which was blocked by AT_4 blockade, but not AT_1 blockade (64,65). Subsequent studies from multiple groups have shown that systemic and intrarenal Ang IV cause vasoconstriction and decrease renal blood flow, the effects of which are inhibited by AT_1 antagonists, but not AT_4 antagonists (66–68). Ang IV has also been implicated in the regulation of cell growth and the vascular inflammatory response in which it stimulates endothelial expression of PAI-1 (69). In vascular smooth muscle cells Ang IV activates the nuclear factor κB (NF-κB) pathway, leading to increased expression of monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), interleukin-6, and TNF-α (70). Ang IV also plays a role in memory and cognition (71).

Angiotensin A (Ang A) and alamandine are two of the more novel peptides in the RAS system. Ang A is an octapeptide generated from the decarboxylation of the first residue of Ang II, Asp^1, to Ala^1 (72). Relative to Ang II, Ang A binds to AT_1 with similar affinity and to AT_2 with slightly greater affinity. As a result, the AT_1-mediated effects of Ang A are not as potent as Ang II. Alamandine, which is formed from Ang A by ACE2 or through decarboxylation of Ang 1-7, has vasoactive effects similar to Ang 1-7 (73).

Angiotensins are also subject to hydrolysis by endopeptidases, the most likely of which to be physiologically relevant is neutral endopeptidase (NEP). NEP directly converts Ang I to Ang 1-7 (74). Ang 1-7 can also be formed from Ang II by prolyl endopeptidase and prolyl carboxypeptidase (75), as well as via cleavage by ACE2. Previously thought to be inactive, Ang 1-7 has been shown to induce renal afferent

arteriolar vasodilatation (76) and to stimulate diuresis and natriuresis, possibly via inhibition of the Na^+/K^+ ATPase in the proximal tubule (77). In addition to its vasodilatory properties, Ang 1-7 decreases cardiac hypertrophy and fibrosis and prevents cardiac remodeling (78,79). These actions appear to be mediated via binding to the G protein-coupled receptor Mas (79,80). The ACE2-Ang(1-7)-Mas axis is thought to be a counterregulatory arm within the RAS, opposing the actions of Ang II (81).

Angiotensin Receptors

The actions of Ang II are mediated through binding to one of the Ang receptor subtypes. The two most well characterized are the type 1 and type 2 receptors, designated AT_1 and AT_2. Both receptor subtypes are G protein-coupled seven transmembrane receptors. Almost all known Ang II effects, including vasoconstriction, aldosterone secretion, increased sympathetic tone, and cellular growth and proliferation, are mediated by the AT_1 receptor, although the functions of the AT_2 receptor are increasingly being unraveled.

The AT_1 receptor has been localized to multiple organs, including the brain, heart, adrenal gland, kidney, and vasculature (82). It is widely distributed throughout the heart, where Ang II binding causes positive inotropy and chronotropy, but receptor density is greatest within the conducting system (83). Ang II binding to AT_1 receptors stimulates aldosterone secretion from the adrenal zona glomerulosa and catecholamine release from the chromaffin cells of the adrenal medulla. The high levels of the receptor throughout the vasculature on smooth muscle cells mediate changes in vascular tone due to Ang II. Within the kidney, AT_1 receptors have been localized to the afferent arteriole, glomerular mesangial cells, renal medullary interstitial cells, vasa recta, and throughout the tubule (84,85). Ang II stimulates sodium and water reabsorption, regulates the glomerular filtration rate (GFR), and inhibits renin secretion from the macula densa. Activation of AT_1 receptors by Ang II stimulates cell growth and proliferation, including activation of the Janus kinases (JAK)/signal transducers and activators of transcription (STAT) pathway (86), expression of growth factors such as TGF-β_1 and basic fibroblast growth factor (bFGF), and vascular smooth muscle cell

and cardiac myocyte hypertrophy (87–89).

The AT_2 receptor seems to oppose AT_1 receptor-mediated effects. It is expressed in cardiac fibroblasts (90), the adrenal medulla (82), renal glomeruli, afferent arterioles, proximal tubule, and vasa recta (85,91). Its abundance in kidney mesenchyme during fetal growth suggests an important role in normal development (92). However, AT_2 receptor-knockout mice develop normally but have altered behavior and cardiovascular function, including an increased pressor response to Ang II infusion (93,94). The AT_2 receptor mediates production of vasodilatory substances in the kidney and induces natriuresis via a cascade involving bradykinin and nitric oxide (95). In contrast to the AT_1 receptor, the AT_2 receptor inhibits cell proliferation and promotes differentiation. Thus, a generally protective role for AT_2 receptors has been suggested. For example, AT_2 receptor-knockout mice suffer greater kidney injury than do wild-type mice in the partial renal ablation model of CKD (96).

Additional Ang receptors have been identified that are distinct from the AT_1 and AT_2 receptors. Ang IV binds with high affinity to the AT_4 receptor but has poor affinity for the AT_1 and AT_2 subtypes. The AT_4 receptor has been localized in multiple organs, including the kidney, heart, central nervous system, and adrenal gland (71). Insulin-regulated aminopeptidase (IRAP) has been proposed as the functional receptor for Ang IV (97), but this has been called into question (71). Data suggest Ang IV may exert its effects through c-Met, a type 1 tyrosine kinase inhibitor (98). The G protein-coupled receptor Mas has been identified as a functional receptor for Ang 1-7, as described earlier. Mas-knockout mice have impaired cardiac function and altered collagen expression toward a profibrotic state (99). The Mas-related G protein-coupled receptor D (MrgD) is the receptor for alamandine. Through the MrgD receptor, alamandine has been shown to have antihypertensive and antifibrotic effects in spontaneously hypertensive rats (SHR) (73).

Angiotensin II

Ang II is the principal effector of the RAS for the regulation of extracellular volume and blood pressure. It acts on multiple organs, including the heart, kidney, vascular system, adrenal gland, central nervous system, and intestine. The effects of Ang II on cardiovascular

function include maintenance of systemic blood pressure via direct constriction of vascular smooth muscle cells, leading to increased systemic vascular resistance (SVR), and enhanced myocardial contractility. Ang II stimulates catecholamine release from the adrenal medulla and sympathetic nerve endings, increases sympathetic nervous system activity, and may enhance the vasoconstrictor response due to catecholamines (100,101). Ang II acts to preserve extracellular volume via increased salt and water retention by stimulating aldosterone secretion from the adrenal glomerulosa, promoting thirst and water intake, and enhancing renal sodium transport.

In the kidney, Ang II directly affects renal hemodynamics, control of GFR, and tubular transport. The actions of Ang II in the kidney have been nicely reviewed by Ichikawa and Harris (102). In summary, Ang II causes arteriolar vasoconstriction, mediated primarily by protein kinase C (PKC) generation (103). It constricts both the afferent and efferent arterioles and the interlobular artery (104–106). Vascular resistance increases more in the efferent than in the afferent arteriole in response to Ang II, partly due to the smaller resting diameter of the efferent arteriole (107). Thus renal blood flow declines and glomerular capillary hydraulic pressure rises, which preserves GFR in the setting of reduced systemic blood pressure.

The vascular actions of Ang II are modulated by other vasoactive substances produced by endothelial cells, vascular smooth muscle cells, and mesangial cells. Vasodilatory prostaglandins and nitric oxide minimize the increase in vascular resistance, while endothelin-1 (108) and metabolites of the lipoxygenase pathway (109) may mediate Ang II-induced vasoconstriction.

Autoregulatory mechanisms are important for maintaining renal blood flow and GFR in a relatively constant range despite large variations in systemic blood pressure. Two primary mechanisms are recognized to maintain autoregulation. Myogenic stretch receptors in the afferent arteriolar wall respond to changes in perfusion pressure manifested as changes in stretch. Alterations in chloride delivery to the macula densa facilitate a response that returns GFR and tubular flow toward normal. This latter effect is called the tubuloglomerular feedback mechanism. Ang II might be expected to play a primary role in the maintenance of autoregulation, but this does not appear to be the case. Rather, Ang II has a permissive influence on TGF, sensitizing the afferent arteriole or other elements to signals from macula densa cells (110). Other vasopressors do not produce a similar response (111).

Ang II exerts direct effects in the proximal tubule to stimulate the

reabsorption of sodium and water, as well as bicarbonate. It increases the activity of the Na^+/H^+ exchanger in the apical membrane of proximal tubule epithelial cells, thereby enhancing Na^+ uptake (112). Ang II stimulates basolateral Na^+/K^+ ATPase activity, further contributing to Na^+ transport (113,114). The Na^+ (HCO_3^-) cotransporter in the basolateral membrane is also activated by Ang II (115). Overall, the effects of Ang II may account for up to 40% to 50% of sodium and water reabsorption in the early (S_1 segment) proximal tubule (116). Ang II also enhances Na^+ reabsorption in the thick ascending limb of the loop of Henle and in the distal tubule (117).

Ang II has effects on cellular growth and proliferation and may contribute to tissue injury in a number of ways (55). It stimulates production of growth factors such as TGF-β and endothelin-1, and it appears to have a role in mediating apoptosis. Ang II induces inflammation via mediators such as NF-κB and MCP-1. These proinflammatory and profibrotic actions partly explain the promotion of glomerulosclerosis and tubulointerstitial fibrosis by Ang II (118).

ACE-Related Carboxypeptidase

The classical notion of the RAS has been extended by discoveries of the ACE-related carboxypeptidase ACE2 and additional Ang peptides with biologic activity. ACE2 protein expression has been demonstrated in the heart, kidney, and testis (119), as well as in epithelia of the human lung and small intestine (120). Like ACE, ACE2 is both a membrane-associated and membrane-secreted enzyme. It generates its main product, the heptapeptide Ang 1-7, via two pathways. ACE2 cleaves the C-terminal Leu residue from Ang I to generate Ang 1-9, the function of which remains unknown. Ang 1-9 is then converted to Ang 1-7 by ACE. Ang 1-7 is also formed by the cleavage of a single residue from Ang II by ACE2. The dual properties of generating the vasodilator Ang 1-7 and degrading Ang II suggest a counterregulatory role for ACE2 in opposing the pressor actions of Ang II (121). Indeed, ACE2-deficient mice show greater pressor sensitivity and higher renal Ang II concentrations during Ang II infusion compared with controls (122). ACE2 may also have an important role in cardiac structure and function (123). In the kidney, ACE2 has been detected in the vascular endothelium, glomerulus, and tubular epithelium (120,124). ACE2-knockout mice develop late glomerulosclerosis that is

prevented by AT_1 receptor blockade, suggesting that the glomerular injury is Ang II dependent (125). Early diabetes may be associated with decreased glomerular expression of ACE2, with greater albuminuria induced by ACE2 inhibition (126). However, the association of ACE2 with diabetic nephropathy remains unclear (127–129).

Local Renin–Angiotensin Systems

It has become clear over the past two decades that the traditional model of the circulating RAS is but one part of the overall picture. There is now abundant evidence of local Ang II synthesis in a variety of sites and the suggestion of complete RASs in several organs. Renin mRNA has been found in multiple tissue sites, although renin secretion has only been demonstrated in the kidney. Ang II production takes place at multiple locations within the kidney, and proximal tubule concentrations of Ang II are 100- to 1,000-fold higher than those in plasma. In addition to Ang II generation within JG cells and the tubulointerstitium, it may be formed in the proximal tubule lumen by membrane-bound ACE. Intraluminal Ang II may then stimulate sodium reabsorption in the distal tubule and collecting duct (55). The full spectrum of Ang-mediated effects may need to be viewed as the sum of local and systemic actions of the RAS. In particular, paracrine and autocrine actions have been proposed for the local RAS with effects at the cellular level regulating such diverse processes as cardiac hypertrophy and fibrosis, vascular inflammation and remodeling, temperature regulation, and behavioral control (130).

Aldosterone

Aldosterone is one of the several steroid hormones produced in the zona glomerulosa of the adrenal cortex. It is the principal steroid regulator of sodium and potassium balance—hence its classification as a mineralocorticoid. Aldosterone is thought to be secreted by the adrenal gland as a result of increased synthesis and simple diffusion of the hormone across the adrenal cell membrane, as no specific membrane carriers have been identified. Circulating aldosterone is about 50% protein bound, largely to albumin. Aldosterone is primarily metabolized in the liver which is one reason its levels rise with liver disease. The kidney

inactivates aldosterone through the formation of physiologically inert glucuronide conjugates and other metabolites that are excreted in the urine. In addition to measuring the plasma level of the active hormone, measurement of the urinary excretion of its metabolites is a common way of assessing aldosterone secretion (131).

STIMULI TO SECRETION

Several factors regulate aldosterone synthesis and secretion by the adrenal gland (Table 8-2) (132). One of the most important of these is Ang II which acts on its G protein-coupled type 1 receptor on the adrenal cell surface to initiate aldosterone synthesis (131). A β arrestin-mediated pathway has also been implicated in transducing Ang II's message in the adrenal gland (133).

Plasma potassium is the second major signal for aldosterone secretion. Small increases in plasma potassium in the range of 0.1 mEq/L can raise plasma aldosterone by 25% (131). While potassium and Ang II independently stimulate aldosterone secretion, they appear to have synergistic effects. For example, when Ang II levels are suppressed by pharmacologic inhibition of the converting enzyme, potassium is less potent in stimulating aldosterone than when Ang II levels are normal. When Ang II is administered exogenously, the full potency of potassium is restored. Thus, some tonic action of Ang II may be necessary for the full effect of potassium (134). This interaction explains the relative failure of potassium alone to maintain aldosterone secretion in patients receiving drugs that inhibit Ang II production or action.

Several other compounds influence aldosterone secretion, including adrenocorticotropic hormone (ACTH), ANP, endothelin, and dopamine (131). The effects of these factors are relatively minor compared to Ang II and potassium.

ACTIONS

Sodium and potassium balance are strongly influenced by aldosterone. Aldosterone enhances sodium reabsorption in the distal nephron through regulation of the epithelial sodium channel (ENaC). Through this and perhaps other tubular effects, aldosterone also augments potassium secretion. Thus, aldosterone acts as a key determinant of extracellular volume and thereby blood pressure as well as plasma potassium levels.

EFFECTS ON SODIUM AND POTASSIUM TRANSPORT

Aldosterone directly and indirectly influences ENaC expression (Fig. 8-2). In the classic model of steroid hormone action, aldosterone binds to a cytoplasmic mineralocorticoid receptor. This complex translocates to the nucleus, which results in increased transcription of target genes such as ENaC subunits (135). Another genomic target of aldosterone is serum- and glucocorticoid-regulated kinase 1 (Sgk1). This enzyme is under transcriptional control by aldosterone (136,137) and is responsible for phosphorylating and thereby inhibiting Nedd4-2, a regulatory protein that promotes ENaC degradation (138,139). Thus, aldosterone also regulates sodium reabsorption by controlling ENaC trafficking. The effect of aldosterone on ENaC is also modulated by pendrin, a Cl^-/HCO_3^- exchange protein localized to the apical membrane of type B and non-A, non-B intercalated cells in the cortical collecting duct. Pendrin, which is upregulated by aldosterone and Ang II, indirectly enhances the abundance and activity of ENaC by increasing luminal bicarbonate concentration and decreasing luminal ATP (140).

Table 8–2 Factors Regulating Aldosterone Secretion

Major Stimuli	Major Inhibitor
Angiotensin II	Atrial natriuretic hormone
Adrenocorticotropin hormone	Hypokalemia
Hyperkalemia	—
Other Stimuli	**Other Inhibitors**
Acetylcholine	Calcitonin gene-related peptide
ATP	Dopamine
Bradykinin	Nitric oxide
Cholecystokinin	Platelet-derived growth factor
β-Endorphin	Somatostatin
Enkephalins	Transforming growth factor-β
Endothelin	Unsaturated fatty acids
Epidermal growth factor	—

12-Hydroxyeicosatetraenoic acid	—
Melanocyte-stimulating hormone	—
Neuropeptide Y	—
Neurotensin	—
Norepinephrine	—
Parathormone	—
Prolactin	—
Prostaglandins	—
Serotonin	—
Substance P	—
Vasoactive intestinal polypeptide	—
Vasopressin	—

The mineralocorticoid receptor has an affinity for aldosterone comparable to its affinity for cortisol. Since cortisol is present in much higher concentrations than aldosterone, the presence of the enzyme 11-*β*--hydroxysteroid dehydrogenase is needed to metabolize cortisol at sites of aldosterone action and thereby allow the actions of aldosterone alone unimpeded by ambient cortisol. This effect is thought to be achieved in part by simple degradation of the cortisol and in part through a reduction in the transcriptional activity of the cortisol–mineralocorticoid receptor complex by an associated rise in NADH.

As noted above, increases in plasma K^+ increase aldosterone secretion (141). The mineralocorticoid has also been shown to increase expression of the renal outer medullary potassium channel (ROMK), which enhances K^+ secretion (142). This effect is synergistic with sodium reabsorption; the latter allows for an increase in luminal negativity in the renal tubules and thereby facilitates K^+ secretion. Aldosterone also acts through Sgk1 to amplify this effect. Sgk1 appears to work in concert with the Na^+/H^+ Exchange Regulating Factor (NHERF2) to increase ROMK activity (143). This finding is supported by the sgk1-knockout mouse, which has impaired renal K^+ clearance (144).

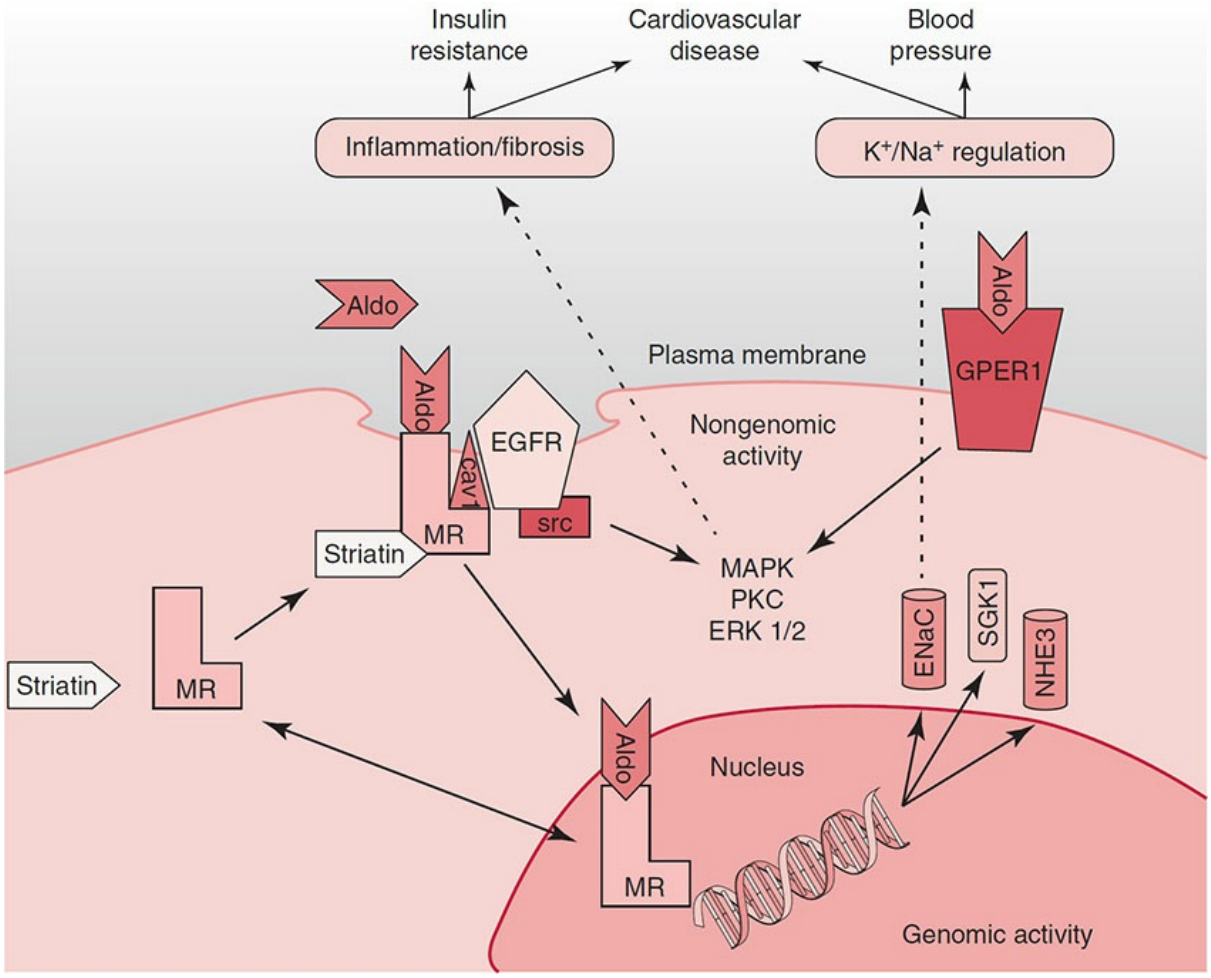

Figure 8–2 Schematic of possible genomic and nongenomic aldosterone actions. Classic, genomic aldosterone (Aldo) action involves Aldo diffusion across the plasma membrane into the cytoplasm where it interacts with the mineralocorticoid receptor (MR) then translocates to the nucleus where it acts as a transcription factor increasing the expression of key regulator channels and enzymes; epithelial sodium channel (ENaC), Na^+/H^+ exchanger (NEH3), serum-induced and glucocorticoid-induced kinase 1 (SGK1), others. Scaffolding proteins, striatin and caveolin-1 (cav1), traffic MR to the cell membrane to interact with aldo and epidermal growth factor receptor (EGFR) via the cytoplasmic tyrosine kinase (src) to trigger nongenomic/secondary messenger cascades (mitogen-activated protein kinase [MAPK], protein kinase C [PKC], extracellular signal-regulated kinase [ERK 1/2]). MR-independent activation of secondary messenger cascades also occurs via aldo binding to the G protein-coupled receptor (GPER1). (Reprinted from Williams JS. Evolving research in nongenomic actions of aldosterone. *Curr Opin Endocrinol Diabetes Obes*. 2013;20:198–203, with permission from Wolters Kluwer Health, Inc.)

In addition to renal epithelia, aldosterone exerts control over ENaC expressed in intestinal, salivary, and sweat epithelial tissue, where it also participates in sodium and potassium homeostasis (145).

A number of other non-hemodynamic pathways of aldosterone action

have been explored. However, several observations give predominance to the ability of aldosterone to raise arterial, and probably glomerular, pressure as the primary mechanism of damage to the kidney and the organism as a whole. The requirement for concomitant high salt intake in most animal models of injury and the absence of tissue injury in forms of secondary aldosteronism without hypertension, such as chronic salt deficiency, are evidence for a key role of hypertension (146,147). Likewise, the development of renal failure in cases of Liddle syndrome, in which aldosterone levels are suppressed, suggests that classic salt-dependent hypertension is very important, regardless of the other aldosterone-dependent pathways that may be at work (148).

NONTRANSPORT EFFECTS

Extra-adrenal sites of aldosterone synthesis have been reported and include the vasculature, kidney, and heart (149–151), the latter of which has been challenged by more recent data (152). However, because the concentration of aldosterone falls to essentially zero after adrenalectomy, it appears that the contribution of extra-adrenal sources of aldosterone to its plasma levels is insignificant. Nevertheless, given the presence of mineralocorticoid receptors in many nonepithelial tissues, including the vasculature and heart, these nontraditional targets may indeed respond to aldosterone whether they produce it or not (153).

Beyond the well-documented effect of aldosterone to expand extracellular volume with the net result of hypertension, direct vascular actions of aldosterone have been proposed. SVR has been reported to change modestly in response to acute aldosterone infusion in normal human subjects (154). Rabbit glomerular afferent and efferent arterioles constrict in response to aldosterone ex vivo (155). In this model, increased calcium flux plays an important role, and calcium channel blockade can inhibit the effect. The direct vascular effects of aldosterone may be complex, involving some interplay with nitric oxide. The homeostatic role of these direct vasoconstrictive actions and their magnitude in vivo are still under investigation.

Beyond raising intravascular pressure, aldosterone may also contribute to injury through stimulation of certain cytokines. The secretion of profibrotic cytokines TGF-β and PAI-1 is provoked by aldosterone (156,157). In addition to these cytokines, several other inflammatory mediators are produced in adipocytes under the influence of aldosterone. Finally, production of reactive oxygen species has been observed in cells

treated with aldosterone. These findings have given rise to the notion that aldosterone contributes to insulin resistance and the metabolic syndrome through inflammatory and oxidant effects.

NONGENOMIC ACTIONS

Aldosterone can change several markers of cellular signaling within minutes. These changes occur well before any increase in protein expression and are not affected by inhibitors of transcription or protein synthesis (158,159). These effects cannot be explained by aldosterone's classic genomic actions and are referred to instead as "nongenomic" actions. Indeed, many of the abovementioned nontransport actions of aldosterone are temporally nongenomic or at least partially so. The molecular biology of aldosterone's nongenomic cell signaling is rapidly evolving and involves complex interactions between protein kinases and second messenger pathways. The paradigm that both the genomic and nongenomic actions of aldosterone require activation of the cytoplasmic mineralocorticoid receptor has been challenged by the discoveries of mineralocorticoid receptors on the plasma membrane (160) and a non-mineralocorticoid G protein-coupled receptor, GPER-1, which has been shown to mediate nongenomic aldosterone signaling (161). Interested readers are referred to elegant reviews of the nongenomic actions of aldosterone by Dooley (162) and Williams (163). While distinct physiologic roles for these nongenomic effects have yet to be determined, it appears that the genomic and nongenomic pathways interact to modulate the overall physiologic effects of aldosterone.

The Renin–Angiotensin–Aldosterone System in Hypertension

The major function of the RAAS is to regulate blood pressure and extracellular volume. Changes in either of these parameters lead to either activation or suppression of the system. However, the appropriate coupling of RAAS activity to blood pressure and volume may be altered in a number of pathologic states. In certain conditions, a primary disturbance in the RAAS leads to abnormalities in blood pressure and/or extracellular volume. These disorders can be distinguished by the relationship between PRA and aldosterone secretion (Table 8-3).

Individuals with hypertension may be characterized by their PRA profile. In approximately one-third of hypertensive subjects, the PRA is below that of normal subjects (called low-renin hypertension). Thus, in the remaining two-thirds, renin secretion is normal or supranormal despite the high-pressure state that would be expected to suppress PRA. Some have advocated the use of PRA measurement to guide treatment in essential hypertension, specifically in those with difficult-to-control hypertension (164,165). This is based on the hypothesis that PRA is a marker for the primary pathophysiologic mechanism, that is, low-renin hypertension is a volume excess state best treated with natriuretic agents; in the remainder, hypertension is mediated by renin-dependent vasoconstriction and should be treated with blockers of the RAAS. However, this approach has not been rigorously tested and is not routine practice for most clinicians.

Primary disorders of the RAAS account for approximately 10% of hypertensive subjects, although prevalence estimates are highly variable depending on the population studied. Mineralocorticoid hypertension is classically accompanied by hypokalemia and metabolic alkalosis. However, this is not universal and should not be considered necessary to raise suspicion for a secondary cause of hypertension. Primary hyperaldosteronism is the most common cause. Disorders of renin secretion and causes of secondary hyperaldosteronism must also be considered (Table 8-3). Unilateral or bilateral vascular lesions of the renal artery stimulate renin release due to decreased renal perfusion pressure. The etiology is most commonly atherosclerotic disease causing renal artery stenosis, especially in older individuals. Fibromuscular dysplasia is the most frequent etiology among younger individuals, presenting most often in women. Renin-secreting tumors are a rare cause of secondary hypertension. Primary hyperaldosteronism may be caused by an adrenal adenoma (Conn syndrome), bilateral adrenal hyperplasia, or, less commonly, an adrenal carcinoma. Glucocorticoid-remediable aldosteronism is an autosomal dominant condition in which a chimeric aldosterone synthase gene results in excess aldosterone secretion under the control of ACTH.

Table 8–3 Differential Diagnosis of Mineralocorticoid Hypertension

High Renin, High Aldosterone
Renovascular hypertension

Renin-secreting tumor
Low Renin, High Aldosterone
Adrenal adenoma
Bilateral adrenal hyperplasia
Adrenal carcinoma
Glucocorticoid-remediable aldosteronism
Low Renin, Low Aldosterone
Apparent mineralocorticoid excess
Liddle syndrome
Pituitary or ectopic ACTH-producing tumor
Congenital adrenal hyperplasia
ACTH, adrenocorticotropic hormone.

Other syndromes may present similarly to hyperaldosteronism, yet are characterized by suppression of aldosterone production. Apparent mineralocorticoid excess (AME) is a rare autosomal recessive disorder marked by inactivity of the enzyme 11-β-hydroxysteroid dehydrogenase. The excess cortisol binds the mineralocorticoid receptor, resulting in sodium retention and volume expansion with suppression of renin and aldosterone. An acquired form of AME may result from licorice ingestion due to the actions of glycyrrhizic acid and glycyrrhetinic acid. Liddle syndrome is caused by an inherited gain-of-function mutation in ENaC, leading to unregulated sodium retention. Ectopic ACTH production and defects in cortisol synthesis may also produce the signs and symptoms of mineralocorticoid excess.

Measurement of the ratio of the plasma aldosterone concentration (measured in ng/dL) to PRA (measured in ng/mL/h) is a useful initial screening test. Values $^{>}25$, particularly with a plasma aldosterone $^{>}15$ ng/mL, suggest primary hyperaldosteronism and should prompt further testing (Fig. 8-3). Interested readers are referred to several excellent reviews of mineralocorticoid hypertension (166); genetic forms of hypertension, including Liddle syndrome (167); primary aldosteronism (168); and syndromes of aldosterone excess and deficiency (169).

Blockade of the Renin–Angiotensin–Aldosterone

RENIN INHIBITORS

Aliskiren is the first member of a new class of orally active, nonpeptide, low-molecular-weight renin inhibitors. Its oral bioavailability is quite low (2.6%), but it is highly potent and has a half-life of 40 hours (170). It interferes with the rate-limiting step in Ang II production, the highly substrate-specific cleavage of angiotensinogen by renin. Aliskiren does not undergo hepatic metabolism and is not metabolized by the cytochrome p450 system. It is primarily excreted in the urine, mostly as unchanged drug.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

ACE inhibitors inhibit the activity of Ang-converting enzyme. There are three categories based on the ligand that binds to the ACE-zinc moiety: sulfhydryl, carboxyl, and phosphinyl. Despite differences in prodrug, structure, binding affinity, and metabolism, the clinical effects of all ACE inhibitors are quite similar. With the notable exceptions of captopril and cilazopril, the duration of response for most is approximately 24 hours. Drug metabolism varies within the class between liver and kidney, although most are excreted at least partially in the urine. The most common side effect is a dry cough that is reported in up to 20% of patients (171). This is mediated by the accumulation of bradykinins and substance P, which are otherwise degraded by ACE, and by the production of prostaglandins. The cough may present immediately after initiation of therapy or several months thereafter. The only established cure is cessation of the drug. The most feared complication of ACE inhibitor therapy is angioedema, which is a potentially life-threatening complication and occurs in approximately 0.3% of patients (172). The mechanism is thought to be related to increased bradykinin levels and inhibition of C1 esterase activity (173).

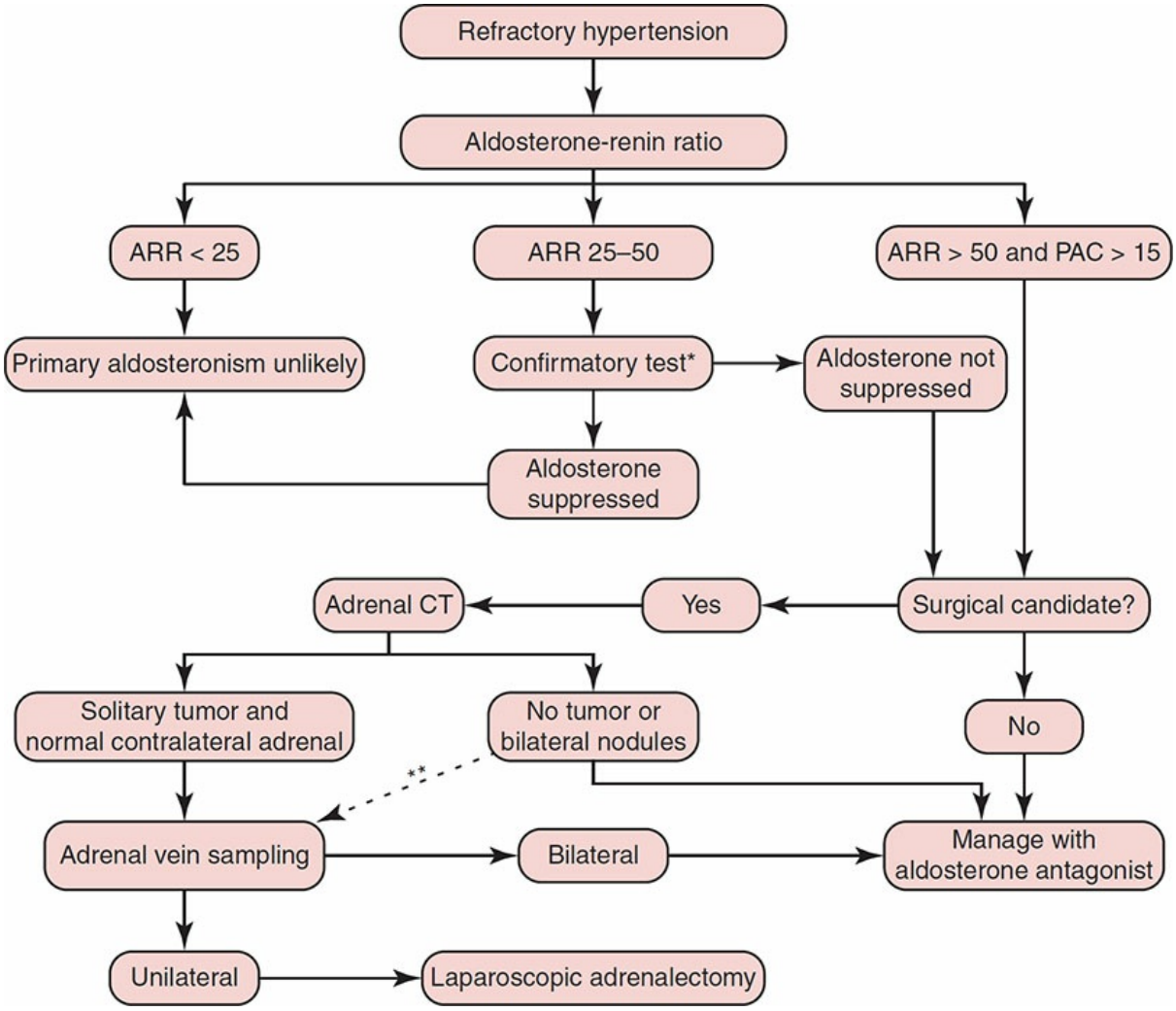

Figure 8–3 Algorithm for the diagnosis of primary aldosteronism. ARR, aldosterone–renin ratio; aldo, aldosterone; CT, computed tomography; PAC, plasma aldosterone concentration. *Confirmatory tests include intravenous saline and fludrocortisone suppression, and captopril challenge. With saline suppression: PAC >10 ng/dL—diagnosis likely; <5 ng/dL—diagnosis unlikely; 5 to 10 ng/dL—indeterminant. With fludrocortisone suppression: PAC >6 ng/dL and plasma renin activity <1 ng/mL/h—diagnosis likely. With captopril challenge: >30% reduction in PAC—diagnosis unlikely. **In the setting of a negative adrenal CT, if blood pressure response to mineralocorticoid antagonism is inadequate, proceeding with adrenal vein sampling may be considered. (Adapted from Funder JW, Carey RM, Fardella C, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metabol*. 2008;93:3266–3281.)

ANGIOTENSIN II TYPE I RECEPTOR ANTAGONISTS

The AT_1 receptor blockers (ARBs) may provide more complete blockade of the RAS than do ACE inhibitors because ARBs interfere with the system at the point of receptor binding by Ang II. Thus, effects of non–

ACE-dependent Ang II formation (due to chymase, cathepsins, etc.; see earlier) are not blocked by ACE inhibitors but are inhibited by ARBs. Like ACE inhibitors, ARBs also increase bradykinin levels. This is due to increased AT_2 receptor activity secondary to lack of Ang II binding to AT_1 receptors. Nevertheless, the incidence of angioedema with ARBs is approximately one-third that of ACE inhibitors and not significantly more than placebo (172).

ARBs also vary in structure, metabolism, potency, and mechanism of receptor inhibition. This class of medication is composed of both peptide and nonpeptide analogs. There are both competitive and noncompetitive antagonists of the AT_1 receptor. Losartan was the first orally active ARB; its derivatives are called biphenyl tetrazoles. Other members of the class are categorized as nonbiphenyl tetrazoles and nonheterocyclic compounds. The duration of response is approximately 24 hours for all. Most ARBs undergo both hepatic and renal metabolism. The incidence of cough in patients with a history of ACE inhibitor-induced cough was no greater than that in controls (174). The incidence of recurrent angioedema with ARBs in patients with a history of ACE inhibitor-induced angioedema is 1.5% to 10% (175,176).

ALDOSTERONE RECEPTOR ANTAGONISTS

Spironolactone and eplerenone are steroid analogues with structural similarity to aldosterone and thereby function as competitive antagonists. Compared to spironolactone, eplerenone is equally potent but more specific for the mineralocorticoid receptor by virtue of a 9,11-epoxy moiety that decreases its binding to androgen and progesterone receptors (177). Both drugs are metabolized hepatically, though spironolactone has multiple active metabolites, whereas eplerenone has none (178). This results in a shorter effective half-life and therefore quicker time to peak response for eplerenone.

As described earlier, these molecules are able to antagonize some, but not all, of the actions of aldosterone. This implies that either aldosterone can signal through a mineralocorticoid receptor distinct pathway or differential mineralocorticoid receptor localization somehow favors access to aldosterone but not spironolactone or eplerenone. As an example of the latter, an open-ring water-soluble aldosterone antagonist, RU28318, completely abrogated the influence of aldosterone on Na^+/H^+ exchanger activity, whereas spironolactone had no effect in a human vascular preparation ex vivo (179). Hyperkalemia is the most serious side effect.

Gynecomastia also occurs and is more frequent with spironolactone.

CKD Progression and the Renin–Angiotensin–Aldosterone System

ACE inhibitors and ARBs are standard drugs for primary hypertension. However, they are each especially effective in slowing the decline of GFR in CKD (180–184). Diabetic nephropathy has been the most studied, and these agents not only lower proteinuria but also slow progressive injury. This general pattern of reduction in proteinuria linked to retardation of filtration failure has been observed in the other major classes of renal injury, including hypertensive nephrosclerosis (185). While these drugs also reduce proteinuria in autosomal dominant polycystic kidney disease and APOL1-associated kidney disease, they have not been shown to mitigate the decline in GFR more than other antihypertensive agents (186,187). The use of RAS antagonists in rarer diseases like Alport syndrome has become routine. This has been primarily based on observational data (188); however, a randomized control trial, EARLY-PRO-TECT, is currently underway (189).

There are several reasons why ACE inhibitors and ARBs are especially beneficial antihypertensive agents in CKD (Fig. 8-4). They reduce Ang II levels and/or action and thereby also lower aldosterone levels. These actions lower arterial pressure, but intrarenal hemodynamic effects also contribute to their salutary effects. In many animal models of CKD, glomerular capillary pressures are elevated and are thought to be elevated in human CKD. ACE inhibitors and ARBs reduce this capillary hypertension both by reducing arterial perfusion pressure and by relaxation of the efferent arteriole, the dominant site of Ang II action. Relief from this excessive capillary pressure likely prevents mesangial cell proliferation and matrix production as well as podocyte loss (190). The associated reduction in proteinuria may also be a benefit of these drugs, as protein absorption by the proximal tubular cells appears to be toxic (191). Finally, as described earlier, Ang II and aldosterone promote several profibrotic and proinflammatory mediators (118,192). Thus, it is both the hemodynamic and anti-fibrotic/inflammatory actions that underlie the efficacy of ACE inhibitors and ARBs in CKD.

Since ACE inhibitors and ARBs each slow progression individually, many questioned whether the combination would provide additional

advantages. An early report of the COOPERATE trial claimed that the combination was superior to the individual drugs (193). However, these results and their analysis have been challenged (194). An analysis of a study designed to examine cardiovascular endpoints in subjects with cardiovascular disease but generally good renal function, the ONTARGET study, found less proteinuria with the combination but no benefit in terms of preventing decline in GFR (195). Use of combination therapy, compared to ACE inhibitor alone, in early or late stage polycystic kidney disease did not slow the decline of eGFR in the HALT-PKD trial (196,197). The VA NEPHRON-D study involving patients with proteinuric diabetic kidney disease was stopped early after combination therapy, compared to ARB alone, increased the risks of hyperkalemia and acute kidney injury without demonstrating a benefit with respect to renal disease progression, cardiovascular events, or mortality (198). At present, the use of combination therapy is not recommended due to the lack of benefit and increased risk of serious adverse events.

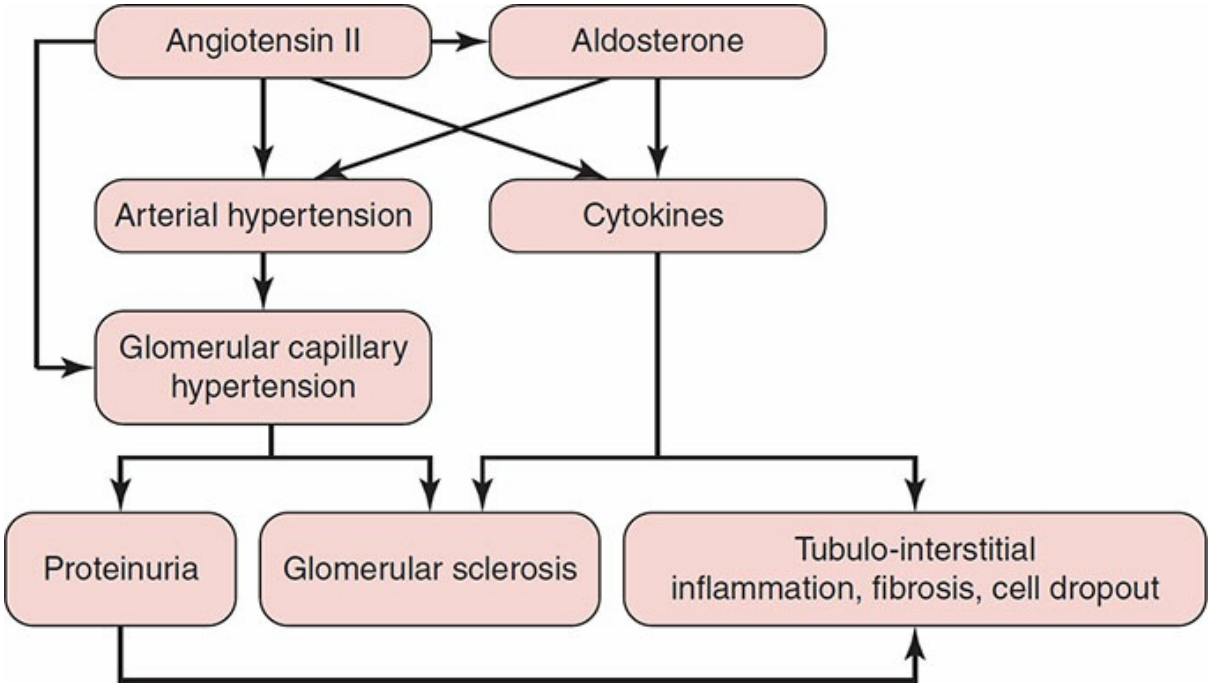

Figure 8–4 RAAS system and renal injury. RAAS, renin–angiotensin–aldosterone system.

Aldosterone contributes along with Ang II to the adverse actions of the RAAS in progressive CKD. Furthermore, an increase in aldosterone with long-term use of ACE inhibitors, ARBs, or renin inhibitors, termed "aldosterone breakthrough," occurs in 30% to 50% of patients (199,200). Recognition of the deleterious effects of aldosterone has led to attempts to

selectively block it using aldosterone receptor antagonists (ARAs) (201). A large number of studies in experimental animals have supported this approach. Several trials in human subjects with CKD have shown a reduction in proteinuria when an ARA was added to an ACE inhibitor or ARB (201). As expected, the risk of hyperkalemia is increased with the addition of an ARA. Two trials designed to determine the effect of adding an ARA to an ACE inhibitor or ARB on cardiorenal endpoints in the CKD population are currently underway (202,203). Currently, there are no firm data to support the use of ARAs in addition to standard therapy in CKD (204).

Inhibition of renin is yet another means of interrupting the RAAS. Addition of a renin inhibitor to an ARB reduced proteinuria, independent of its effect on blood pressure, in subjects with diabetic nephropathy (205). However, a larger and longer trial on the effects of adding a renin inhibitor to an ACE inhibitor or ARB was stopped early due to lack of benefit in terms of renal disease progression and cardiovascular events, and an increased risk of hyperkalemia (206).

In summary, blockade of the RAAS with ACE inhibitors or ARBs has proven effective in slowing the progression of CKD, in particular diabetic kidney disease. While inhibiting multiple sites of the RAAS decreases proteinuria independent of blood pressure, such practice is not recommended due to the lack of cardiovascular and renal benefits, and the increased risk of serious adverse events.

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CHAPTER 9

The Kidney in Hypertension

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Global Burden of Hypertension

Cardiovascular disease is the most common cause of death in economically developed countries and is rapidly evolving as a major cause of morbidity and mortality in economically developing nations as well (1). Certainly, hypertension is among the most important modifiable risk factors and the leading risk factor for disease burden worldwide (2). On the basis of currently recommended criteria for diagnosis of hypertension (systolic blood pressure [SBP] ≥140 mm Hg or diastolic blood pressure [DBP] ≥90 mm Hg, or use of antihypertensive medication), approximately one-third of men and women have hypertension in the United States (3). The overall prevalence of hypertension has not changed since 1999 to 2000 (4). Recent changes to Joint National Committee (JNC) 8 proposed less restricted guidelines for BP targets in individuals with hypertension (5). Despite this, BP goals were met in less than half of the young adults and two-thirds of older adults in the United States (6). There is a progressive rise in SBP throughout life, with a difference of 20 to 30 mm Hg between early and late adulthood. Likewise, DBP tends to rise with age up until the fifth decade; in later decades DBP declines. These patterns result in a progressively higher prevalence of hypertension with aging, which consists of predominant elevation of SBP or isolated systolic hypertension. A majority of adults have hypertension by the sixth decade

and >70% have it by the seventh and eight decades of life. Among normotensive individuals in their sixth decade, the lifetime risk of developing hypertension approaches 90%. African American adults have an incidence and prevalence of hypertension that is 50% higher than their white or Mexican American counterparts. The average level of BP and prevalence of hypertension also increased progressively in children and adolescents between 1988 and 2000. The prevalence of hypertension in many other countries is as high as or higher than that identified in the United States. Estimates by the World Health Organization (WHO) suggest that approximately 40% of the adults have hypertension, with the highest prevalence in Africa estimated at approximately 46% (7). In the last few decades, the prevalence of hypertension in economically developing countries appears to have risen; in 2008, 1 billion individuals had uncontrolled hypertension (7). BP exhibits a dose-dependent relationship with the risk of cardiovascular disease throughout the entire range of BP in the population. The relationship is independent of other cardiovascular risk factors with no evidence of a BP threshold for risk. The BP risk applies to all major manifestations of cardiovascular disease including stroke, sudden cardiac death, coronary heart disease, heart failure, aortic aneurysm, peripheral vascular disease, as well as chronic kidney disease (CKD) and end-stage renal disease (ESRD). Although elevations of both SBP and DBP are independently associated with cardiovascular risk, high SBP is the most potent predictor of risk (8,9). Population-attributable risk estimates had indicated that attainment of a population average SBP <115 mm Hg would reduce the occurrence of ischemic heart disease by 50% and stroke by 60%, thereby preventing about 7 million deaths annually on a worldwide basis (1).

Historical Perspective: The Link between Hypertension and Renal Dysfunction

The occurrence of hypertension in the setting of renal disease and its impact on the progression of renal insufficiency has long been of interest to clinicians. The concept that hypertension is in some way related to renal dysfunction was first proposed by Bright in 1836 (10). He recognized the association between hypertrophy of the heart and contraction of the kidney and postulated that the cause was increased cardiac work required to force blood through a vascular tree constricted by irritating humoral substances

that accumulate in renal failure. The role of fluid retention in the genesis of renal hypertension was first outlined by Traube in 1871 (11), who proposed that with shrinkage of the renal parenchyma, a decreasing amount of fluid is removed from the arterial system by urinary secretion, thereby resulting in hypertension.

Mahomed in 1879 (12) was the first to clearly describe hypertension of unknown cause, without evidence of underlying renal disease (now called essential hypertension). He emphasized that the most frequent complications in individuals with this type of hypertension were cardiovascular and most often occurred in the absence of significant renal dysfunction. However, in 1914, Volhard and Fahr (13) defined a subgroup of patients with essential hypertension who eventually developed severe renal involvement. They distinguished two types of hypertensive nephrosclerosis, benign and malignant. The benign type, characterized by hyaline arteriolosclerosis, was associated with a slowly progressive course with eventual complications caused by heart failure or stroke in the absence of clinically significant renal impairment. In contrast, malignant nephrosclerosis was characterized by arteriolar necrosis and endarteritis that resulted in rapidly progressive renal failure and death. Volhard (14) subsequently introduced the concept of the vicious circle in which renal disease causes hypertension, which in turn exacerbates renal injury.

Over the years, it has been recognized that the kidney is both “villain and victim” in hypertension (15). The kidney, even when histologically normal, is felt to play a central role in the pathogenesis of essential hypertension. Molecular genetic studies have identified mutations in eight genes that cause mendelian forms of hypertension (16). Each of these genetic defects that impart very large effects on BP leads to enhanced renal tubular sodium reabsorption (impaired natriuresis), resulting in salt-sensitive hypertension. Recently, two coding sequence variants in apolipoprotein L1 (ApoL1), G1 and G2, have been identified as an important risk factor for progressive nondiabetic kidney disease associated with hypertension in African Americans (17). In addition, other underlying primary renal parenchymal disease or abnormalities of the renal vasculature can cause secondary hypertension. On the other hand, the kidney may also suffer the brunt of hypertension. Essential hypertension that enters a malignant phase can rapidly destroy the kidney. Furthermore, recent evidence suggests that hypertension is a major factor in the progression of CKD in the setting of both diabetic and nondiabetic CKD.

We address three major questions with regard to the interaction between the kidney and hypertension. What role does the kidney play in

the genesis of essential hypertension and the various forms of secondary hypertension? Why does hypertension develop in the setting of primary renal disease? What is the role of treatment of hypertension in slowing the progression of CKD?

Circulatory Hemodynamics: Complexity in a Simple Relationship

At face value, the physiology of BP regulation is deceptively simple, behaving according to Ohm's law whereby the mean arterial pressure (MAP) is defined by the product of the cardiac output times the systemic vascular resistance. Thus, hypertension can only result from an increase in either of these two variables. As such, the kidney is expected to have a major influence on BP given its central role in the regulation of extracellular fluid (ECF) volume. An increase in ECF volume caused by renal sodium and water retention should cause increased blood volume, venous return, and filling pressure, which in turn should increase stroke volume and cardiac output via the Frank–Starling mechanism and result in hypertension. Volume expansion, resulting from excess sodium intake or an underlying abnormality in renal sodium excretory mechanisms, has been considered to be an important mechanism for the development of hypertension in animal models as well as in humans with essential hypertension or CKD.

The paradox, which we attempt to explain, is that a significant increase in ECF volume or cardiac output is difficult to demonstrate in human essential hypertension as well as animal models, at least in the chronic phase. Chronic essential hypertension is virtually always maintained by an increase in systemic vascular resistance arising primarily at the level of precapillary arterioles throughout the circulatory system (18). This observation has formed the basis for the widely held concept that an increase in systemic vascular resistance is the underlying primary cause of hypertension, and many theories exist regarding the roles of various vasoconstrictor mechanisms (19) or absence of vasodilator substances (20).

However, there are complexities in the seemingly simple equation that relates BP to cardiac output and systemic vascular resistance. The circulatory system is dynamic and a perturbation, such as ECF volume expansion, which initially leads to hypertension via an increase in cardiac

output, may result in compensatory hemodynamic responses that ultimately restore sodium balance and thereby normalize ECF volume and cardiac output. This restoration of sodium balance results from a “pressure natriuresis” caused by systemic hypertension that is maintained by a chronic increase in systemic vascular resistance (21–25). The various theories proposed to account for the phenomenon whereby initially volume-dependent hypertension evolves into high systemic vascular resistance hypertension are described in detail in the following sections.

Dietary Salt and Hypertension

Archeological studies of Paleolithic man suggest that the diet of these hunter-gatherers was very low in sodium and relatively high in potassium. Sodium intake averaged 30 mmol/day with a ratio of dietary potassium to sodium of 16:1 (26). Because total body sodium content is the major determinant of ECF volume, a very efficient renal sodium conservation mechanism evolved in the face of this limited availability of sodium. In contrast, the modern urban diet contains 120 to 300 mmol sodium per day (24) and only 65 mmol potassium per day (27). Therefore, the human species today is faced with a much higher daily sodium load than that to which it adapted over roughly 2 million years (26). Because virtually all ingested sodium is absorbed, sodium intake in excess of insensible losses must be excreted by the kidney. Under normal circumstances, even in the face of tremendous dietary sodium excess, humoral and other mechanisms cause an increase in urinary sodium excretion with little increase in ECF volume (28). However, this natriuretic mechanism is aberrant in a segment of the population, so that excessive dietary salt intake results in the development of hypertension (21–25,29).

EPIDEMIOLOGIC AND CLINICAL STUDIES

Studies throughout the world have suggested a correlation between the mean dietary sodium intake and prevalence of hypertension in the population. In parts of Japan where the mean sodium intake is >400 mmol/day, the prevalence of hypertension approaches 50% (30). In contrast, in certain inland populations where the sodium intake is very low (0.2–51 mmol/day), hypertension is virtually nonexistent and there is no tendency for the BP to rise with age (31). The INTERSALT study investigated the relation between dietary sodium intake (defined by 24-

hour urine sodium excretion) and BP in 10,000 subjects in 32 countries (32). A significant positive correlation was found between sodium intake and BP even when the data were adjusted for age, gender, body weight, and alcohol consumption. The data suggest that habitually high sodium intake (>150 mmol sodium per day) is a critical environmental factor that contributes to the high prevalence of hypertension in most urban populations, whereas lifelong ingestion of a diet extremely low in sodium (<50 mmol/day) prevents the development of hypertension (30,31). Extremely high sodium intake (>800 mmol sodium per day) has been shown to raise the BP of healthy normotensive individuals (33). On the other hand, diets with <10 mmol sodium per day have been shown to lower the BP of most hypertensive patients (34). Additional support for the importance of dietary sodium in the genesis of hypertension is the finding that, in an adult population, long-term lowering of the sodium intake was associated with a fall in the prevalence of hypertension and a concomitant reduction in cerebrovascular mortality (35).

The effect of dietary composition on BP is a subject of major public health importance. The Dietary Approaches to Stop Hypertension (DASH) diet study demonstrated that a diet that emphasizes on fruits, vegetables, and low-fat dairy foods; includes whole grains, poultry, fish, and nuts; contains only small amounts of red meat, sweets, and sugar-containing beverages; and has reduced amounts of total and saturated fat and cholesterol substantially lowers BP as compared with the typical diet in the United States (36). In a subsequent report, the effect of different levels of dietary sodium intake, in conjunction with the DASH diet, was studied in subjects with and without hypertension (37). A total of 412 participants were randomly assigned to eat either a control diet typical of intake in the United States or the DASH diet. Within each assigned diet, participants ate foods containing high sodium (150 mmol/day, typical consumption in the United States), intermediate sodium (100 mmol/day, as in the no-added salt diet), and low sodium (50 mmol/day) for 30 consecutive days each, in random order. The DASH diet was associated with significantly lower SBP at each sodium intake level. Moreover, the reduction in sodium intake significantly lowered SBP and DBP in a stepwise fashion, with both the control and DASH diets. Reducing dietary sodium intake had approximately twice as great an effect on BP with the control diet as it did with the DASH diet. Reducing the sodium intake from high to intermediate level reduced the SBP by 2.1 mm Hg ($P < 0.001$) during the control diet and by 1.3 mm Hg ($P = 0.03$) during the DASH diet. Reducing sodium intake from intermediate to low level caused additional reductions

of 4.6 mm Hg during the control diet ($P < 0.001$) and 1.7 mm Hg during the DASH diet ($P < 0.01$). Dietary sodium restriction resulted in a greater reduction in BP in subjects with hypertension than in normotensive subjects. As compared with the control diet with high sodium intake, the DASH diet with low sodium intake led to a mean SBP that was 7.1 mm Hg lower in participants without hypertension and 11.5 mm Hg lower in participants with hypertension. The effects of dietary sodium on BP were observed in participants with and without hypertension, African Americans, and those of other races, and in both men and women. On the basis of the levels of dietary sodium intake actually achieved in the study, it appears that BP can be reduced in individuals consuming either a diet that is typical in the United States or the DASH diet by reducing sodium intake from approximately 140 mmol/day (average sodium intake in the United States) to an intermediate level of 100 mmol/day (the currently recommended no-added salt diet), or from this level to a still lower level of 65 mmol/day (equivalent to 1.5 g sodium or 3.8 g sodium chloride). The impact of these findings on public health of course depends on the ability of people to make long-lasting dietary modifications and increased availability of lower sodium foods in the marketplace.

The Genetic Epidemiology Network of Salt-Sensitivity (GenSalt) study demonstrated that nondiabetic individuals with metabolic syndrome and underlying insulin resistance have increased sensitivity of BP to changes in dietary sodium intake (38). In GenSalt, 1,906 Chinese participants without diabetes, ages 16 years or more, were selected to receive a low-sodium diet (51.3 mmol/day) for 7 days followed by a high-sodium diet (308.8 mmol/day) for an additional 7 days. BP was measured at baseline and on days 2, 5, 6, and 7 of each intervention. Metabolic syndrome was defined as the presence of three or more of the following: abdominal obesity, raised BP, high triglyceride concentration, low high-density lipoprotein (HDL) cholesterol, or high glucose. High salt sensitivity was defined as a decrease in MAP of >5 mm Hg during low-sodium diet or an increase of >5 mm Hg during the high-sodium diet. Overall 283 (28%) of subjects met criteria for diagnosis of metabolic syndrome. Multivariable-adjusted mean changes in BP in response to changes in dietary sodium intake were significantly different in subjects with and without the metabolic syndrome. There was a significant and graded association between the number of risk factors for metabolic syndrome and age-adjusted and gender-adjusted proportion of study participants with high salt sensitivity of BP. Compared with those with no risk factors for the metabolic syndrome, participants with four or five risk

factors had a 3.54-fold increased odds of high salt sensitivity during the low-sodium intervention and a 3.13-fold increased odds of high salt sensitivity after the high-sodium diet. Moreover, compared with participants without the metabolic syndrome (less than three risk factors), participants with metabolic syndrome (three or more risk factors) had a 92% increased odds of high salt sensitivity after the low-sodium diet and a 70% increased odds of high salt sensitivity after the high-sodium intervention. The association between metabolic syndrome and salt sensitivity of BP was independent of age, gender, body mass index, physical activity, cigarette smoking, alcohol consumption, and baseline dietary intake of sodium and potassium. Furthermore, the association between metabolic syndrome and salt sensitivity remained significant even if participants with baseline hypertension were excluded. As discussed in more detail later, insulin resistance and concomitant compensatory hyperinsulinemia may lead to renal sodium retention (impaired natriuresis) and thereby explain the BP response to changes in dietary sodium intake in individuals with metabolic syndrome. Data from the GenSalt study suggest that reduction in dietary sodium intake may be an especially important strategy for reducing BP in an individual with multiple risk factors for the metabolic syndrome.

On balance, it is clear that sodium intake must play a permissive role in the development of hypertension, because a lifelong diet very low in sodium prevents hypertension (31). On the other hand, excessive sodium intake alone is not sufficient to cause hypertension, because the majority of individuals on such a diet fail to develop hypertension. These observations imply that there must be additional predisposing factors that lead to the development of hypertension in certain individuals when the intake of sodium is >60 to 70 mmol/day.

DIETARY SALT AND ANIMAL MODELS OF GENETIC HYPERTENSION

When groups of rats were maintained on a wide range of dietary sodium, the mean BP in each group was directly related to sodium intake. Dahl noted that at each level of dietary sodium intake, only some rats became hypertensive. By selective inbreeding, he was able to show that the predisposition to develop hypertension was genetically determined, and he produced a salt-sensitive strain that develops hypertension on a high-sodium diet and a salt-resistant strain that remains normotensive (39).

The Role of the Kidney in Essential Hypertension

In *kidney cross-transplantation experiments* between four different strains of genetically hypertensive rats (Dahl salt-sensitive hypertensive rats, Milan hypertensive rats, spontaneously hypertensive rats [SHR], and stroke-prone spontaneously hypertensive rats [SHRSP]), and their respective normotensive control strains, it was found that BP determinants were carried within the kidney (40). Thus, transplantation of a kidney from a hypertensive strain rat into a bilaterally nephrectomized rat from the normotensive strain results in hypertension in the recipient. Conversely, transplantation from a normotensive strain rat into a nephrectomized hypertensive strain rat prevents the development of hypertension. Thus, the BP of the recipient rat is dependent on the source of the donated kidney. On the other hand, it could be argued that the posttransplant hypertension in recipients of kidneys from hypertensive strains is not caused by a primary defect in the donor kidney, but instead results from hypertension-induced changes in the donor kidney and that these acquired secondary structural defects in the kidney lead to hypertension in the recipient. To address this question, the development of hypertension in the SHRSP kidney donors was prevented by chronic antihypertensive drug treatment (40). Despite sustained normalization of BP in the donor rats, the recipients developed posttransplant hypertension. This finding indicates that SHRSP kidneys carry a primary defect that can elicit hypertension. These experiments suggest that the predisposition to genetic hypertension resides in the kidney and is not determined directly by systemic humoral abnormalities or changes in vascular reactivity that have been described in these models. The latter abnormalities must represent either epiphenomena or secondary changes in response to a primary renal abnormality.

These animal models of hypertension bear a remarkable resemblance to human essential hypertension. Indeed, studies of kidney transplant patients also support a primary role for the kidney in the development of essential hypertension. In patients with essential hypertension and renal failure caused by malignant nephrosclerosis, bilateral native nephrectomy in conjunction with a well-functioning renal allograft, from a normotensive cadaver donor, cures essential hypertension (41). In a study of six such patients, before renal transplantation, MAP was 168 ± 9 mm Hg despite treatment with a minimum of a four-drug antihypertensive regimen. However, following bilateral native nephrectomy and successful renal transplantation, at a mean follow-up of 4.5 years, MAP without antihypertensive treatment was 92 ± 1.9 mm Hg. Another observation that

suggests a role for the kidney in the pathogenesis of hypertension is the finding that the incidence of hypertension in recipients of cadaver kidneys correlates with the incidence of essential hypertension in the family of the donor (42). These intriguing reports support the notion that the defect that causes human essential hypertension resides within the kidney.

Pathogenetic Mechanisms of Impaired Natriuresis

If the relationship between sodium intake and hypertension represents cause and effect, then it is important to explain why high sodium intake leads to hypertension in only some individuals. In this regard, it has been postulated that in the setting of essential hypertension in humans or in salt-sensitive animal models, there is a genetically predetermined impairment in the renal ability to excrete sodium (21–25). This postulated renal abnormality has been termed an "unwillingness to excrete sodium" or "impaired natriuretic capacity." In studies of isolated, perfused kidneys from Dahl salt-sensitive strains, at age 8 weeks, even before the onset of hypertension, there is a defect in natriuresis such that at any given perfusion pressure, less sodium is excreted in comparison with kidneys from salt-resistant strains (43). In humans, the heritability of essential hypertension has been well established in epidemiologic surveys. The prevalence of hypertension among offspring has been reported to be 46% if both parents are hypertensive, 28% if one parent is hypertensive, and only 3% if neither parent is hypertensive (44). The familial aggregation of hypertension is not simply attributable to shared environmental effects because adoption studies show greater concordance of BP among biological siblings than adoptive siblings living in the same household (45). Moreover, twin studies document greater concordance of BP between monozygotic twins than dizygotic twins (46). Analysis of the natriuretic response to slow infusion of saline has revealed that normotensive first-degree relatives of patients with essential hypertension excrete a sodium load less well than control subjects without a family history of hypertension (47). Among blacks and individuals over 40 years old (48)—two groups with an increased incidence of hypertension—studies of normotensive individuals also have demonstrated a slower natriuretic response to saline infusion than in controls, suggesting that a diminished natriuretic capacity may underlie the predisposition to essential hypertension in these groups.

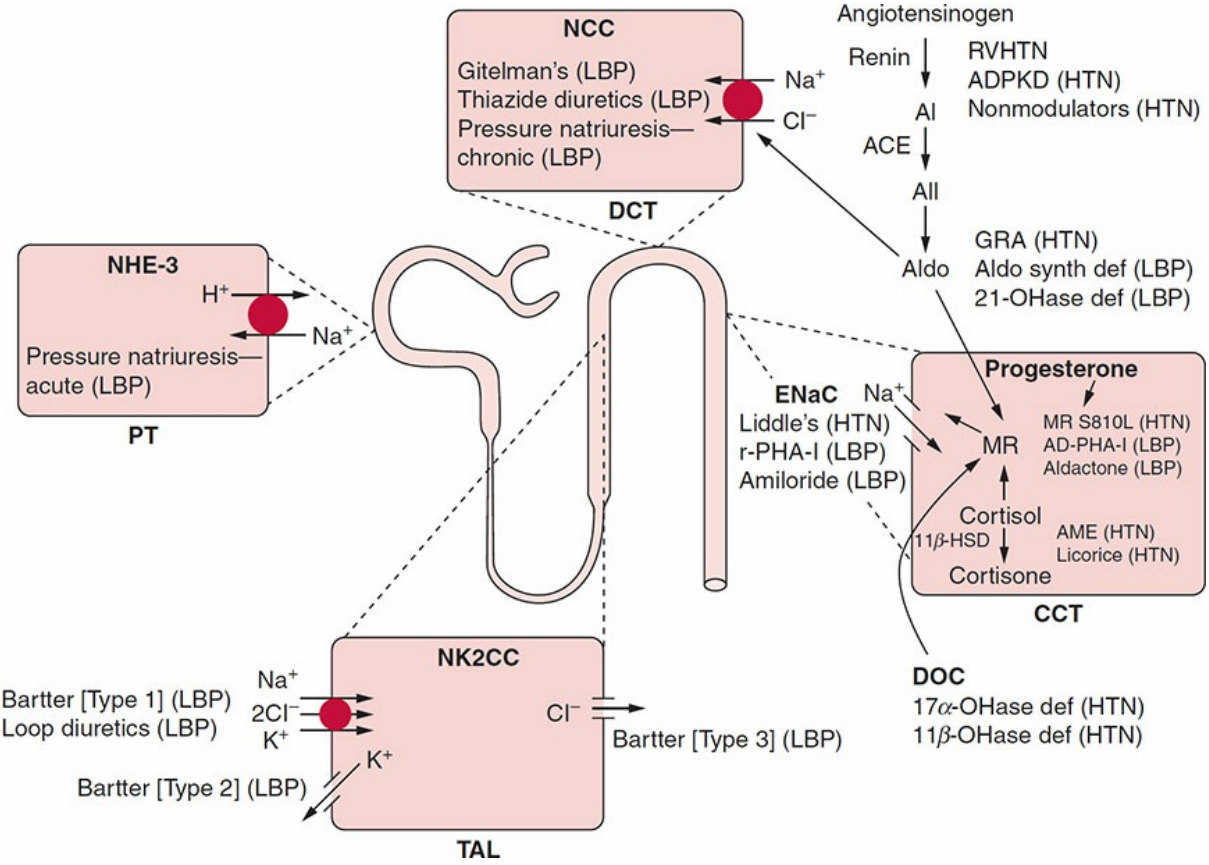

Figure 9-1 Alterations in renal sodium handling that influence systemic blood pressure (BP). This diagram of the nephron illustrates the molecular pathways mediating sodium reabsorption in individual cells in the proximal tubule (PT) (Na^+/H^+ exchanger, NHE-3); the thick ascending limb (TAL) of the loop of Henle (Na/K/2Cl cotransporter, NK2CC); the distal convoluted tubule (DCT) (thiazide-sensitive Na/Cl cotransporter, NCC); and cortical collecting duct (amiloride-sensitive epithelial sodium channel, ENaC, the activity of which is regulated by the mineralocorticoid receptor, MR). Also shown is the pathway of the renin–angiotensin–aldosterone system (RAAS), the major regulator of renal salt reabsorption, sodium balance, and BP. Various inherited diseases, medical disorders, and pharmacologic agents that influence renal sodium handling and BP are listed adjacent to the molecular pathways they involve in Figure 9-1. Conditions that enhance renal sodium reabsorption and therefore cause hypertension are labeled *HTN*. Conditions and drugs that impair renal sodium reabsorption and thereby lower blood pressure are labeled *LBP*. In the PT, downregulation of NHE-3 mediates acute pressure natriuresis. In the TAL, three different variants of Bartter syndrome result from various autosomal recessive loss-of-function mutations that impair sodium reabsorption and lower blood pressure. Type 1 results from mutation in the $Na^+/K^+/2Cl$ cotransporter, which is also the transporter inhibited by loop diuretics. Type 2 results from mutation in the ROMK channel, which is crucial for recycling of potassium from the cell into the lumen to maintain efficiency of the NK2CC. Type 3 results from mutation of the basolateral chloride channel, which is responsible for the exit of reabsorbed chloride from the cell. In the DCT, Gitelman syndrome results from autosomal recessive loss-of-function mutation in the NCC, which is also the molecular site of action of thiazide diuretics. Chronic pressure natriuresis is mediated by

downregulation of the abundance of the NCC. In the DCT, Liddle syndrome results from gain-of-function mutations in the ENaC leading to enhanced sodium reabsorption and hypertension. ENaC is blocked by the potassium-sparing diuretic amiloride. Loss-of-function mutations in ENaC occur in the autosomal recessive form of pseudohypoaldosteronism type 1 (PHA1). The MR S810L mutation in the MR leads to an autosomal dominant form of hypertension that is markedly exacerbated during pregnancy because the mutant receptor is activated by progesterone. MR is inhibited by spironolactone, another potassium-sparing diuretic. Loss-of-function mutations in the MR cause autosomal dominant PHA 1. In the syndrome of apparent mineralocorticoid excess (AME), loss-of-function mutations in 11-*b*-hydroxysteroid dehydrogenase (11*b*-HSD) prevent metabolism of cortisol to cortisone so that cortisol binds and activates the MR leading to enhanced sodium reabsorption and hypertension. Natural licorice causes hypertension by inhibiting 11*b*-HSD. Cushing syndrome and ectopic secretion of ACTH by certain tumors lead to markedly elevated cortisol levels that overwhelm 11*b*-HSD, allowing cortisol to bind to MR, leading to enhanced sodium reabsorption and hypertension. Glucocorticoid remediable hyperaldosteronism (GRA) causes hypertension as the result of a chimeric gene that drives constitutive overexpression of aldosterone under the influence of ACTH. Aldosterone synthase deficiency and 21-hydroxylase deficiency (21-OHase def) result in hypotension because of the inability to produce aldosterone and other 21-hydroxylated mineralocorticoids. The adrenogenital syndromes result from deficiencies of 17*a*-hydroxylase (17*a*-OHase) or 11*b*-hydroxylase (11*b*-OHase), which result in overproduction of the mineralocorticoid hormone deoxycorticosterone (DOC), resulting in enhanced renal sodium reabsorption and hypertension. Increased activity of the renin–angiotensin–aldosterone axis may mediate salt-sensitive hypertension in renovascular hypertension (RVHTN), autosomal dominant polycystic kidney disease (ADPKD), and a subset of patients with essential hypotension who fail to downregulate the RAAS during salt loading (nonmodulators). Additional abbreviations: AI, angiotensin I; ACE, angiotensin-converting enzyme; AII, angiotensin II; HTN, hypertension; LBP, low blood pressure. (Reprinted from Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104(4):545–556, 2001, with permission from Elsevier.)

NORMAL RENAL SODIUM HANDLING

On a daily basis, the kidneys filter >170 L of plasma containing 23 mol of sodium. Therefore, in an individual consuming a 2-g sodium diet containing 100 mEq of sodium, maintenance of sodium homeostasis requires that the kidneys reabsorb 99.5% of the filtered sodium load. This efficient process of renal sodium reabsorption is accomplished by a complex integrated array of sodium exchangers, sodium transporters, and sodium ion channels. Along the entire length of the nephron, the driving force for sodium reabsorption is the Na^+/K^+ ATPase located in the basolateral membrane of tubular cells, which extrudes sodium from the cell into the blood-side of the tubule and maintains low intracellular

sodium concentration. The distinct functional properties of various portions of the nephron are determined by differences in the sodium transporters located in the apical membrane (Fig. 9-1). Sixty percent of the filtered sodium is reabsorbed in the proximal tubule (PT), largely by the Na^+/H^+ exchanger (NHE-3) and to a lesser extent by the sodium phosphate cotransporter (NaPi-2). Thirty percent of filtered sodium is reabsorbed in the thick ascending limb (TAL) of Henle by the Na/K/2Cl cotransporter. Seven percent is reclaimed by the thiazide-sensitive Na/Cl cotransporter (NCC) in the distal convoluted tubule (DCT). The remaining 2% to 3% is reabsorbed via the epithelial sodium channel (ENaC) in the cortical collecting tubules. Although ENaC accounts for only a small fraction of total renal tubular sodium reabsorption, this is the principal site for regulation of net sodium balance because the activity of this channel is highly regulated by the renin–angiotensin–aldosterone system (RAAS). Decreased perfusion pressure in the afferent arteriole or decreased delivery of sodium to the TAL leads to secretion of renin, an aspartyl protease that acts on angiotensinogen produced in the liver to produce angiotensin I (AI). Through the action of angiotensin-converting enzyme (ACE) in the lung and elsewhere, AI is converted to angiotensin II (AII). AII binds to its specific G protein-coupled receptor in the zona glomerulosa of the adrenal gland, leading to increased secretion of aldosterone, the principal mineralocorticoid steroid hormone. The actions of aldosterone are mediated chiefly by binding to intracellular nuclear hormone mineralocorticoid receptors (MRs) that function as transcription factors when they are in the activated state (49). Aldosterone stimulates sodium retention by the kidney in part through its action to regulate the ENaC, which mediates apical sodium entry across principal cells in the collecting tubules (50). Aldosterone also stimulates sodium reabsorption in the DCT by increasing the abundance of the thiazide-sensitive NCC (51). Thus, both NCC and ENaC appear to be primary targets for the regulation of sodium excretion by aldosterone.

MOLECULAR MECHANISMS IMPLICATED IN BLOOD PRESSURE VARIATION IN HUMANS

Landmark molecular genetic studies by Lifton et al. recently have identified a substantial number of genes in which rare mutations impart large effects on BP leading to various mendelian forms of hypertension or hypotension (16). Investigation of the genetic causes of hypertension or hypotension has provided major insights into the pathophysiologic

mechanisms that can lead to hypertension. Given the vast array of physiologic systems that can influence BP, it is striking that, in all of the mendelian forms of hypertension and hypotension discussed in detail in the following sections, the fundamental underlying abnormality has been found to involve alternations in renal sodium handling (Fig. 9-1). These findings clearly establish the central role of altered sodium homeostasis in the pathogenesis of hypertension and underscore the pivotal role of the kidney in the long-term regulation of BP. Unfortunately, genetic studies in the general population thus far have been disappointing in that no genetic variants that have a substantial effect on BP have been identified. Nonetheless, the finding that all known inherited and acquired forms of hypertension converge on the same final common pathway leading to impaired natriuresis suggests that the pathophysiologic disorders that lead to essential hypertension in the general population will ultimately be found to result directly or indirectly from abnormalities in renal sodium handling.

BLOOD PRESSURE VARIATIONS CAUSED BY MUTATIONS AFFECTING CIRCULATING MINERALOCORTICOID HORMONE

The major regulator of ENaC activity is the MR and its steroid hormone ligand aldosterone. A number of genetic disorders leading to hypertension or hypotension result from abnormalities in aldosterone secretion or the production of other steroid hormones that activate the MR. *Glucocorticoid remediable aldosteronism* (GRA) is an autosomal dominant trait with the phenotype of early-onset hypertension with normal or elevated plasma aldosterone despite suppressed plasma renin activity (PRA) (Fig. 9-1) (52). Hypokalemia and metabolic alkalosis are present in some patients. The hallmark of this disease is normalization of BP during treatment with exogenous glucocorticoids, which completely suppresses the overproduction of aldosterone. GRA is caused by a gene duplication arising from an unequal crossover between the gene encoding for steroid 11β-hydroxylase (an enzyme involved in cortisol biosynthesis that contains an adrenocorticotropic hormone [ACTH] response element) and the gene encoding for aldosterone synthase (the rate-limiting enzyme in aldosterone biosynthesis in the adrenal glomerulosa). The resulting chimeric gene encodes for a protein with aldosterone synthase enzymatic activity whose expression is regulated by ACTH. The net result is that aldosterone synthase is ectopically expressed in the adrenal fasciculate under the control of ACTH rather than AII, the normal hormonal regulator.

Aldosterone secretion thereby becomes linked to cortisol secretion such that maintenance of normal cortisol levels leads to constitutive aldosterone oversecretion, enhanced ENaC activity with increased distal tubular sodium reabsorption, volume expansion, and hypertension. The expanded plasma volume suppresses PRA but fails to suppress aldosterone production. Exogenous administration of glucocorticoid suppresses normal ACTH production and abrogates the ectopic production of aldosterone with normalization of BP.

There are several genetic disorders in which steroids other than aldosterone cause activation of the MR, leading to impaired natriuresis and salt-sensitive hypertension. The *syndrome of apparent mineralocorticoid excess* (AME) is an autosomal recessive disorder that causes early-onset hypertension with hypokalemic metabolic alkalosis (53). The PRA is suppressed and circulating aldosterone is absent. In normal individuals, circulating cortisol levels are 1,000-fold higher than aldosterone levels. In vitro, cortisol is known to bind and activate the MR. However, in vivo, virtually all MR activation is mediated by aldosterone. This paradox is explained by the finding that in the kidney MR specificity for aldosterone is mediated indirectly by the enzyme 11-β-hydroxysteroid dehydrogenase (11β-HSD). In the ENaC-containing principal cells of the collecting duct, expression of 11β-HSD results in metabolism of cortisol to cortisone, which is not capable of activating the MR. Thus, cortisol does not have a mineralocorticoid effect in normal individuals. However, in patients with AME, the absence of 11β-HSD allows cortisol to bind and activate the MR, resulting in salt-sensitive hypertension owing to ENaC-mediated enhancement of tubular sodium reabsorption. The development of hypertension following chronic ingestion of large amounts of *natural licorice* shares a similar pathogenesis. A licorice metabolite, glycyrrhetinic acid, is a potent inhibitor of 11β-HSD, resulting in a phenocopy of AME. Likewise, overproduction of cortisol caused by adrenal adenoma, ACTH-producing pituitary adenoma, or ectopic ACTH production, can overwhelm normal 11β-HSD activity so that cortisol is available for binding and activation of the MR, resulting in salt-sensitive hypertension with hypokalemic metabolic alkalosis.

Other genetic disorders are also associated with excess mineralocorticoid activity, resulting in chronic hypertension. In the adrenogenital syndromes, inherited autosomal recessive *11-*^{b}*-hydroxylase deficiency* (54) and *17*^{a}*-hydroxylase deficiency* (55) lead to impaired cortisol biosynthesis with compensatory hypersecretion of ACTH, which diverts steroid synthesis into pathways proximal to the enzymatic block.

As a result, there is overproduction of 21-hydroxylated steroids such as deoxycorticosterone (DOC) and corticosterone, which are potent activators of the MR, leading to ENaC overexpression, enhanced sodium reabsorption in the DCT, and severe salt-sensitive hypertension (Fig. 9-1). The hypertension responds to cortisol replacement in these disorders, which suppresses ACTH and mineralocorticoid overproduction.

In contrast to disorders associated with excess mineralocorticoid that cause salt-sensitive hypertension, genetic disorders that impair aldosterone synthesis lead to mendelian forms of hypotension. Individuals homozygous for *aldosterone synthase deficiency* (16) have the phenotypic mirror image of GRA, with renal salt wasting and impaired secretion of K^+ and H^+ in the distal nephron (Fig. 9-1). These individuals present with severe hypotension caused by reduced intravascular volume with hyperkalemic metabolic acidosis. Likewise, homozygous *21-hydroxylase deficiency* (56) results in the absence of circulating aldosterone, leading to volume depletion and hypotension (Fig. 9-1).

BLOOD PRESSURE VARIATIONS CAUSED BY MUTATION IN THE MINERALOCORTICOID RECEPTOR

A mutation in the ligand-binding domain of the MR causes an autosomal dominant form of hypertension that develops before the age of 20 years and increases markedly in severity during pregnancy (57). Carriers of this *mineralocorticoid receptor missense mutation (MR S810L)* develop hypertension at a young age. Steroids lacking 21-hydroxyl groups, such as progesterone, normally bind but fail to activate the MR. However, in carriers of the MR S810L missense mutation, progesterone binds and functions as a potent activator of MR. Because progesterone levels rise 100-fold during pregnancy, it is not surprising that all pregnancies among women harboring this mutation have been complicated by the development of severe pregnancy-induced hypertension accompanied by complete suppression of the RAAS.

In contrast to gain-of-function mutations of the MR, which cause hypertension, loss-of-function mutations in the MR cause renal salt wasting and hypotension. There are both autosomal dominant and autosomal recessive forms of the disease. *Autosomal dominant pseudohypoaldosteronism type 1* (PHA1) is characterized by neonatal salt wasting with hypotension despite markedly elevated aldosterone levels in association with hyperkalemic metabolic acidosis (Fig. 9-1). Affected kindred have heterozygous loss-of-function mutation of the MR due to

various premature terminations or frameshift mutations (58). The resulting partial loss of MR function impairs maximal sodium reabsorption, leading to salt wasting and hypotension. Insufficient ENaC activity causes a diminution in the electrical driving force for H^+ and K^+ secretion resulting in hyperkalemia and metabolic acidosis. In the neonatal period, two normal copies of the MR are apparently required for normal sodium homeostasis because with consumption of a normal salt-rich diet, older affected individuals become normotensive with resolution of the biochemical abnormalities.

BLOOD PRESSURE VARIATIONS CAUSED BY ALTERATIONS IN RENAL SODIUM CHANNELS AND TRANSPORTERS

Mutations leading to a gain-of-function mutation in the ENaC also cause salt-sensitive hypertension. *Liddle syndrome* is characterized by autosomal dominant transmission with early-onset hypertension in association with hypokalemic metabolic alkalosis, suppressed PRA, and low plasma aldosterone levels. This disease is caused by mutations in either the β or γ subunit of the ENaC in which there is deletion of their cytoplasmic carboxy termini (59). These mutations result in enhanced ENaC activity that is attributable to an increase in the number of ENaC inserted into the luminal membrane of principal cells in the DCT. The enhanced number of channels is caused by reduced clearance of ENaC from the cell membrane that substantially prolongs ENaC half-life. The increase in ENaC activity leads to enhanced sodium reabsorption, increase in net renal sodium balance, and salt-sensitive hypertension. The pivotal role of impaired natriuresis in the pathogenesis of hypertension is further illustrated by a case report in which severe hypertension in a patient with Liddle syndrome was cured by successful kidney transplantation from a normotensive donor (60). The most common ENaC variant is the *T594M* mutation, which causes a gain-of-function in the β-subunit, and has been found among some individuals with essential hypertension. In a case–control study, 206 hypertensive and 142 normotensive blacks who lived in London were screened for the T594M mutation. It was found that 17 (8.3%) of the hypertensive participants compared with 3 (2.1%) of the normotensive participants possessed the T594M variant (61). These findings suggested that the T594M mutation could contribute to secondary essential hypertension in black people.

In contrast to gain-of-function mutations in ENaC that cause

hypertension, loss-of-function mutations result in hypotension. *Autosomal recessive* PHA1 is caused by loss-of-function mutation in any one of the three ENaC subunits (Fig. 9-1). This disorder causes life-threatening renal salt wasting and hypotension, with hyperkalemic metabolic acidosis despite elevated aldosterone levels (62). Like the autosomal dominant form of PHA1, this disorder begins in the neonatal period; however, it does not resolve on consumption of a salt-rich diet. Affected individuals require lifelong treatment with massive salt supplementation and treatment for hyperkalemia.

To date, hypertension resulting from gain-of-function mutations in the sodium transporters in the PT, TAL, or DCT has not been reported. However, loss-of-function mutations in the Na/K/2Cl cotransporter in the TAL and the thiazide-sensitive NCC in the DCT have been well characterized (16). These autosomal recessive disorders are associated with low–normal BP in association with hypokalemic metabolic alkalosis. In all cases, the disease is caused by mutations that result in renal sodium wasting. Affected individuals can present in the neonatal period with life-threatening hypotension owing to renal sodium wasting or can have disease that is found incidentally. The *Gitelman syndrome* results from homozygous loss-of-function mutations in the thiazide-sensitive NCC (Fig. 9-1) (63). Renal salt wasting leads to BP that is lower than in the general population. Patients present in adolescence with neuromuscular symptoms resulting from hypokalemia. Like thiazide diuretic-treated patients, they have hypomagnesemia and hypocalciuria. The salt wasting at the level of DCT leads to compensatory activation of the RAAS. Activation of the MR leads to augmentation of ENaC activity, thereby enhancing sodium reabsorption at the expense of increased H^+ and K^+ secretion leading to hypokalemic metabolic alkalosis. Linkage studies in a large Gitelman kindred indicate that loss-of-function mutations in the NCC indeed lower BP, thereby providing convincing evidence that even a modest enhancement of the natriuretic capacity of the kidney causes a reduction in BP (64). The *Bartter syndrome* occurs in individuals homozygous for various loss-of-function mutations in any of the three genes required for normal function of the Na/K/2Cl cotransporter in the TAL (Fig. 9-1) (16,65). Impaired function of the transporter leads to marked renal salt wasting, reflex activation of the RAAS, and hypokalemic metabolic alkalosis (Fig. 9-1) (16). In type I Bartter syndrome, the mutation may reside in the Na/K/2Cl cotransporter. In type II, the defect resides in the ATP-sensitive K^+ channel ROMK, which is required for potassium recycling and efficient reabsorption of Na^+ and K^+ in the TAL.

Type III is caused by homozygous mutation in the chloride channel (CLCNKB), which is required for chloride exit from the cell across the basolateral membrane (16). Individuals with Bartter syndrome often present with premature delivery and life-threatening hypotension caused by salt wasting in the neonatal period. In contrast to Gitelman syndrome, Bartter syndrome is associated with hypercalciuria and normal or only slightly reduced magnesium levels (similar to patients treated with loop diuretics).

Gain-of-function mutations in the sodium–hydrogen ion exchanger (NHE-3) in the PT have been investigated as a possible cause of essential hypertension in the general population; however, linkage analysis studies have failed to demonstrate an association (66).

IMPAIRED NATRIURESIS CAUSED BY HYPERINSULINEMIA

It has been observed that glucose intolerance, hyperlipidemia, and essential hypertension tend to cluster in the same patients. In fact, essential hypertension often occurs many years before the onset of overt diabetes with hyperglycemia. It has been proposed that insulin resistance is central to the pathogenesis of this so-called "syndrome X," a condition now known as metabolic syndrome (67). Insulin resistance, which may be inherited or acquired (owing to obesity, dietary factors, or sedentary life style), results in compensatory *hyperinsulinemia*. Eventually, the β-cell output of insulin may become inadequate to compensate for insulin resistance, resulting in glucose intolerance or frank type 2 diabetes. The hyperinsulinemia induces abnormalities of lipid metabolism with increased low-density lipoprotein and reduced HDL levels. Hyperinsulinemia also may be causally related to the development of hypertension. Insulin has been shown to play an important role in renal sodium handling. In human studies in which euglycemic hyperinsulinemia was generated using an insulin clamp technique, urinary sodium excretion declined significantly within 60 minutes (68). This antinatriuretic effect of insulin was observed in the absence of changes in glomerular filtration rate (GFR), renal plasma flow, the filtered load of glucose, or plasma aldosterone concentration (PAC). The predominant effect of insulin on tubular sodium reabsorption is in more distal parts of the nephron (TAL of Henle or DCT). Thus, the net effect of insulin resistance and resulting hyperinsulinemia is to induce an impairment in the intrinsic natriuretic capacity of the kidney, which results in the development of salt-sensitive hypertension.

ACQUIRED TUBULOINTERSTITIAL DISEASE IN THE PATHOGENESIS OF SALT-DEPENDENT HYPERTENSION

There is compelling evidence that essential hypertension results from a defect whereby the kidney is unable to maintain sodium homeostasis at a normal BP. However, if the defect that causes impaired natriuresis is genetic, then why does hypertension generally not develop until adulthood? The salt-sensitive hypertension associated with obesity and aging appears to be an acquired disorder. Why does salt sensitivity increase in frequency and magnitude over time? It has been proposed that salt-sensitive hypertension may be the result of acquired tubulointerstitial renal disease (69). The hypothesis is that hypertension consists of two phases. The first phase is characterized by episodic elevations in BP caused by hyperactivity of the sympathetic nervous system (elevated plasma norepinephrine [NE] and baroreceptor sensitivity) induced by genetic or environmental factors. Less commonly, the episodic hypertension relates to activation of the renin–angiotensin system (RAAS). Elevations of BP are episodic and renal sodium handling is normal during this initial phase. Transition to the second phase is proposed to occur as a consequence of transient catecholamine or AII-mediated elevations in BP that preferentially damage the juxtamedullary and medullary regions, which do not autoregulate, as well as cortical regions in response to sudden changes in renal perfusion pressure. The pressor response may be associated with both an increase in peritubular capillary pressure and a reduction in peritubular capillary flow, resulting in injury to the peritubular capillaries with ischemia of the tubules and interstitium. The resulting tubulointerstitial injury then may trigger local vasoconstrictor mechanisms (AII, adenosine, or renal sympathetic nerves) or inhibit vasodilator mechanisms (nitric oxide [NO], prostaglandins, or dopamine), further augmenting ischemia and resulting in abnormal tubuloglomerular feedback and enhanced tubular sodium reabsorption. Peritubular capillary damage and rarefaction lead to an increase in renovascular resistance, which further blunts the pressure natriuresis mechanism. The predicted consequence of enhanced tubuloglomerular feedback and impaired pressure natriuresis is an acquired functional defect in renal sodium excretion. This resetting of the pressure natriuresis curve to higher pressure is proposed to be the explanation for the development of acquired salt-sensitive hypertension.

Animal models confirm that short-term exposure to catecholamines or AII causes renal injury, which leads to the development of salt-sensitive

hypertension that persists even after catecholamine and AII infusions are stopped (70,71). An 8-week infusion of phenylephrine by mini pump was found to induce structural and functional changes in the kidneys of rats (70). Glomeruli were spared but focal tubulointerstitial fibrosis was present in association increased expression of transforming growth factor-β (TGF-β), de novo expression of the macrophage adhesive protein osteopontin by injured tubules, macrophage and α-smooth muscle actin-positive myofibroblast accumulation in the interstitium, as well as distortion and rarefaction of the peritubular capillaries. BP returned to normal in rats maintained on low-salt diet after discontinuation of catecholamine infusion. However, rats fed on a high-salt diet developed marked hypertension. Short-term (2-week) infusion of AII also caused sustained salt-sensitive hypertension in association with tubulointerstitial damage and fibrosis (71).

This hypothesis may help to explain the development of salt-sensitive hypertension in some high-risk populations. For instance, the prevalence of salt-sensitive hypertension increases progressively with age in the general population. Aging is associated with a progressive decline in renal function and the development of glomerulosclerosis and interstitial fibrosis. In rat models, aging is associated with tubulointerstitial fibrosis characterized by tubular injury, myofibroblast proliferation, osteopontin expression, macrophage infiltration, and collagen IV deposition (72). These structural changes could lead to impaired natriuresis with the development of salt-sensitive hypertension. Obesity also is associated with an increased prevalence of hypertension. Obese individuals have been shown to have augmented sympathetic nervous system activity with higher basal NE and plasma renin levels, glomerular hyperfiltration, increased prevalence of interstitial fibrosis and glomerulosclerosis, expanded ECF volume, and salt-dependent hypertension (69). In transplant patients, treatment with cyclosporine A (CsA) to prevent allograft rejection is also associated with interstitial fibrosis, osteopontin and TGF-β expression, and the development of salt-sensitive hypertension in humans (69). Cyclosporine is a vasoconstrictor that can directly inhibit NO production. Rats administered CsA develop interstitial fibrosis identical to that observed in humans with tubulointerstitial injury preferentially involving the juxtaglomerular regions. Interstitial fibrosis develops in association with TGF-β and osteopontin expression and reduction in endothelial NO synthase. If CsA administration is stopped after the tubulointerstitial lesion develops, placement of the animals on a high-salt diet results in rapid development of hypertension despite the presence of normal GFR (69).

IMPAIRED NATRIURESIS CAUSED BY REDUCED NEPHRON MASS

It has been postulated that the underlying cause of impaired natriuretic capacity in essential (genetic) hypertension may be a congenitally acquired deficit of effective nephron mass (29). In the SHR, the fenestrae of the glomerular capillary endothelium are smaller than in normotensive control rats. In the Milan SHR, there is a decreased glomerular ultrafiltration coefficient and increased proximal sodium reabsorption. Salt-sensitive hypertension in rats is associated with a 15% reduction in nephron number. In humans, major inborn deficits of nephron number such as oligomeganephronia and congenital unilateral renal agenesis are associated with the development of hypertension. Brenner has postulated that the abnormality that predisposes a minority of the population to essential hypertension in the setting of excessive sodium intake is an inherited deficit of nephrons or glomerular filtration surface area leading to a diminished capacity to excrete a sodium load resulting in salt-sensitive hypertension (29). Of course, CKD of any etiology could also lead to a state of impaired natriuretic capacity with resulting salt-sensitive hypertension.

IMPAIRED NATRIURESIS CAUSED BY ABNORMAL REGULATION OF THE RENIN–ANGIOTENSIN SYSTEM

Increased circulating levels of AII stimulate increased production of aldosterone with activation of the MR and enhanced sodium reabsorption in the distal nephron as discussed in detail in the preceding section. AII has also been shown to increase renal sodium and water retention independent of its effect to increase aldosterone production (73). In this regard, AII-mediated renal hemodynamic changes lead indirectly to increases in tubular sodium reabsorption. Infusion of AII causes a reduction in renal blood flow with maintenance of near-normal GFR because of an increase in filtration fraction consistent with an increase in efferent arteriolar resistance. The increase in efferent arteriolar resistance caused by AII results in a fall in hydrostatic pressure and an increase in oncotic pressure in the peritubular capillaries of the proximal and distal tubules and collecting ducts, which results in enhanced tubular sodium reabsorption (73). AII also has been shown to directly stimulate sodium reabsorption in the PT (74).

Recent evidence suggests that renal abnormalities involving disordered

regulation of the RAAS may be important in the pathogenesis of essential hypertension (75). These fundamental abnormalities, which are present in 45% of patients with essential hypertension, cause impairment in renal sodium handling resulting in sodium-sensitive hypertension. These patients have normal to high PRA and have been termed “nonmodulators” because of their inability to appropriately adjust activity of the RAAS during changes in sodium intake. Renal blood flow changes in parallel to sodium intake in normal individuals or patients with essential hypertension and who are “modulators.” Renal blood flow rises with an increase in sodium intake, whereas renal blood flow declines with a decrease in sodium intake. In contrast, the renal blood flow remains fixed in nonmodulators despite changes in sodium intake. This abnormal renal vascular response to changes in sodium intake may account for the limited capacity of the kidney to handle a sodium load. Nonmodulators demonstrate less suppression of renin in response to sodium loading or AII infusion compared with normal individuals or modulators with essential hypertension. Treatment of these nonmodulators with an ACE inhibitor restores the normal natriuretic response to a sodium load and normalizes renin suppression in response to a sodium load or AII infusion. Furthermore, ACE inhibition also results in a decrease in renal vascular resistance and an increase in renal blood flow and natriuresis (75). These findings suggest that enhanced renal vascular responsiveness to AII is present in nonmodulators, even in the absence of increased systemic renin and AII levels, perhaps secondary to in situ AII production by renal-converting enzyme. The resulting decrease in peritubular Starling force and increase in proximal and distal sodium reabsorption may underlie the defect in natriuresis in some cases of essential hypertension. Moreover, restoration of the ability of the kidney to handle a sodium load may explain why treatment with ACE inhibitors leads to a reduction in BP in patients with low-renin essential hypertension.

SYMPATHETIC NERVOUS SYSTEM-MEDIATED IMPAIRMENT IN NATRIURESIS

Neural mechanisms activated in response to perceived changes in BP or intravascular volume act on the kidney to modulate renal sodium excretion. Proximal tubular sodium reabsorption is enhanced by α-adrenergic receptor-mediated sympathetic activation (76). Increased vasoconstrictor responsiveness of the efferent arteriole to α-adrenergic stimuli may cause the intrarenal hemodynamic abnormalities typical of

patients with essential hypertension, namely decreased renal blood flow, increased renal vascular resistance, and increased filtration fraction (77). Akin to the effect of AII, this primary increase in efferent resistance may contribute to the defective natriuresis in essential hypertension by altering peritubular capillary Starling forces. In addition, increased α-adrenergic activity has been shown to directly stimulate sodium reabsorption in the PT (78). Calcium channel blockers (CCBs) decrease renal efferent arteriolar vasoconstriction and improve renal blood flow (77). The natriuretic response to CCBs may be secondary to this phenomenon in addition to their direct effect on tubular sodium transport (79).

The renal nerves contribute to development of hypertension in experimental models, and appear to play a role in the pathogenesis of hypertension in humans (80). In both the SHR and DOC-salt rat models of hypertension, the full development of hypertension is dependent on renal afferent nerve activity, which stimulates renal tubular sodium reabsorption. Denervation of the kidney ameliorates hypertension in these models (80).

IMPAIRED NATRIURESIS CAUSED BY ABNORMALITIES IN RENAL NITRIC OXIDE

There is increasing evidence that endothelium-derived NO is tonically synthesized within the kidney and that NO plays a crucial role in the regulation of renal hemodynamics and sodium excretion (81). These effects are mediated in part by interactions between NO and the RAAS. NO is an important mediator of renal blood flow and the renal microcirculation. Bradykinin and acetylcholine induce renal vasodilation by increasing NO synthesis, which in turn leads to enhancement of diuresis and natriuresis. Blockade of basal NO synthesis, with specific inhibitors of the L-arginine NO pathway (L-NAME or L-NMMA), has been shown to result in an increase in renal vascular resistance with decreases in renal blood flow, urine flow, and sodium excretion. Intrarenal inhibition of NO synthesis leads to a reduction in sodium excretory responses to changes in renal perfusion pressure without an effect on renal autoregulation, suggesting that NO exerts a permissive or mediatory role in the tubular responses that regulate the pressure natriuresis mechanism (82,83). NO released from the macula densa may modulate tubuloglomerular feedback response by affecting afferent arteriolar constriction. In the collecting duct, an NO-dependent inhibition of solute transport has been suggested. Although most studies indicate that NO synthesis blockade causes reduction in sodium excretion, the exact mechanism is unclear. Reduction

in the filtered load of sodium, increased tubular sodium reabsorption, altered medullary blood flow, and decreased renal interstitial hydrostatic pressure may mediate sodium retention during NO blockade. Taken together, these observations suggest that NO-dependent mechanisms have a major impact on renal volume control. Abnormalities in renal NO-mediated sodium excretion have been postulated to cause a state of impaired natriuresis, resulting in salt-sensitive hypertension (81–83). In this regard, impaired NO synthase activity related to insulin resistance may contribute to the pathogenesis of salt-sensitive hypertension in patients with the metabolic syndrome (84).

IMPAIRED NATRIURESIS CAUSED BY ENHANCED PROXIMAL TUBULAR SODIUM REABSORPTION

In some cases, the genetic defect that underlies the development of essential hypertension may be a diminished natriuretic capacity caused by a primary increase in PT sodium reabsorption (85). Lithium clearance often is used as a surrogate marker of proximal tubular sodium reabsorption because lithium reabsorption occurs in parallel to sodium resorption and is limited to the PT. Therefore, a decreased fractional lithium clearance (ratio of lithium clearance to creatinine clearance) implies increased proximal sodium reabsorption. Studies in patients with essential hypertension have revealed a decreased fractional lithium clearance (85). Furthermore, normotensive subjects with a hypertensive first-degree relative had a lower fractional lithium clearance than subjects with no hypertensive relative (85).

IMPAIRED NATRIURESIS: A PREREQUISITE IN HYPERTENSION

Experiments with isolated, perfused kidneys demonstrate that the magnitude of urinary sodium excretion is a direct function of the perfusion pressure (86). The level of perfusion pressure may alter sodium excretion by changing the peritubular hydrostatic pressure. Thus, an increase in perfusion pressure should increase peritubular hydrostatic pressure with a resultant decrease in sodium reabsorption. Micropuncture studies in the rat have shown an inverse relationship between renal perfusion pressure and proximal sodium reabsorption (87).

It has been argued that if this pressure natriuresis mechanism were operating in a normal fashion, then profound volume depletion would

occur in the setting of hypertension. The fact that this does not occur suggests that in every hypertensive state, there must be a shift in the pressure natriuresis curve such that a higher perfusion pressure is required to achieve any given level of natriuresis. In this regard, it has been postulated that this shift in the pressure natriuresis curve is actually a reflection of the underlying renal abnormality present in essential hypertension as well as in hypertension caused by CKD (21,24,25). If a primary defect in natriuresis does exist in hypertension, then to avert disaster owing to persistent positive sodium balance with inexorable fluid accumulation, compensatory hormonal responses or other mechanisms must be invoked that restore sodium balance. The theories regarding the pathogenesis of hypertension that follow explain how these compensatory processes restore sodium balance but in the process cause systemic hypertension in association with an elevated systemic vascular resistance.

The Na/K ATPase Inhibitor Hypothesis

There must be an underlying abnormality in the kidney's ability to excrete sodium in both essential hypertension and secondary hypertension caused by renal disease. In individuals with this impairment of natriuretic capacity, if the intake of dietary sodium is >60 mmol/day, then there will be a tendency toward sodium and water retention resulting in ECF volume expansion. Some authors have proposed that in response to this expansion of ECF volume, there is an increase in the plasma concentration of two or more substances, collectively referred to as "natriuretic hormone" (21,22,88,89). The responses induced by this natriuretic hormone include an increase in the natriuretic capacity of the plasma, an increase in the ability of the plasma to inhibit Na/K ATPase, and an increase in vascular responsiveness to vasoconstrictors such as NE, AII, and vasopressin. Atrial natriuretic peptide (ANP), which is released from the atria in response to acute volume expansion, causes a brisk increase in renal sodium and water excretion of rapid onset and short duration. However, ANP does not inhibit Na/K ATPase or increase vascular reactivity; in fact, ANP decreases systemic vascular resistance. It is proposed that another response to the underlying renal impairment in the ability to excrete sodium is an increase in the plasma concentration of a substance, probably of hypothalamic origin, which inhibits Na/K ATPase (90,91). As a result of inhibition of Na/K ATPase, renal tubular sodium reabsorption is reduced and urinary sodium excretion increases, thereby returning sodium balance

toward normal. However, this circulating inhibitor also inhibits the sodium pump in other cells, such as erythrocytes and leukocytes and, more importantly, in vascular smooth muscle cells. The increase in cellular sodium is associated with increased Na/Ca exchange and increased cellular calcium concentration. Thus, at the arteriolar level, inhibition of Na/K ATPase theoretically could cause vasoconstriction secondary to increased intracellular calcium with a resultant increase in systemic vascular resistance and a rise in BP (88). As a result of the compensatory release of these natriuretic substances, sodium balance and ECF volume are restored to normal but at the expense of systemic hypertension caused by the increase in systemic vascular resistance (Fig. 9-2). Thus, although the underlying cause of this type of salt-sensitive (volume-dependent) hypertension is a defect in renal natriuretic capacity, this does not result in a detectable increase in ECF volume or cardiac output in the steady-state phase. Instead, the hypertension is maintained by the resulting increase in systemic vascular resistance (21,22,88,89).

The Guyton Hypothesis

Guyton's hypothesis is that the most important and fundamental mechanism determining the long-term control of BP is the *renal fluid–volume feedback mechanism*. In simple terms, this is the basic mechanism through which the kidneys regulate arterial pressure by altering renal excretion of sodium and water, thereby controlling circulatory volume and cardiac output. Changes in BP, in turn, directly influence renal excretion of sodium and water, thereby providing a negative feedback mechanism for control of ECF volume, cardiac output, and BP. The hypothesis is that derangements in this renal fluid–volume pressure control mechanism are the fundamental cause of virtually all hypertensive states (21,23–25,92–94).

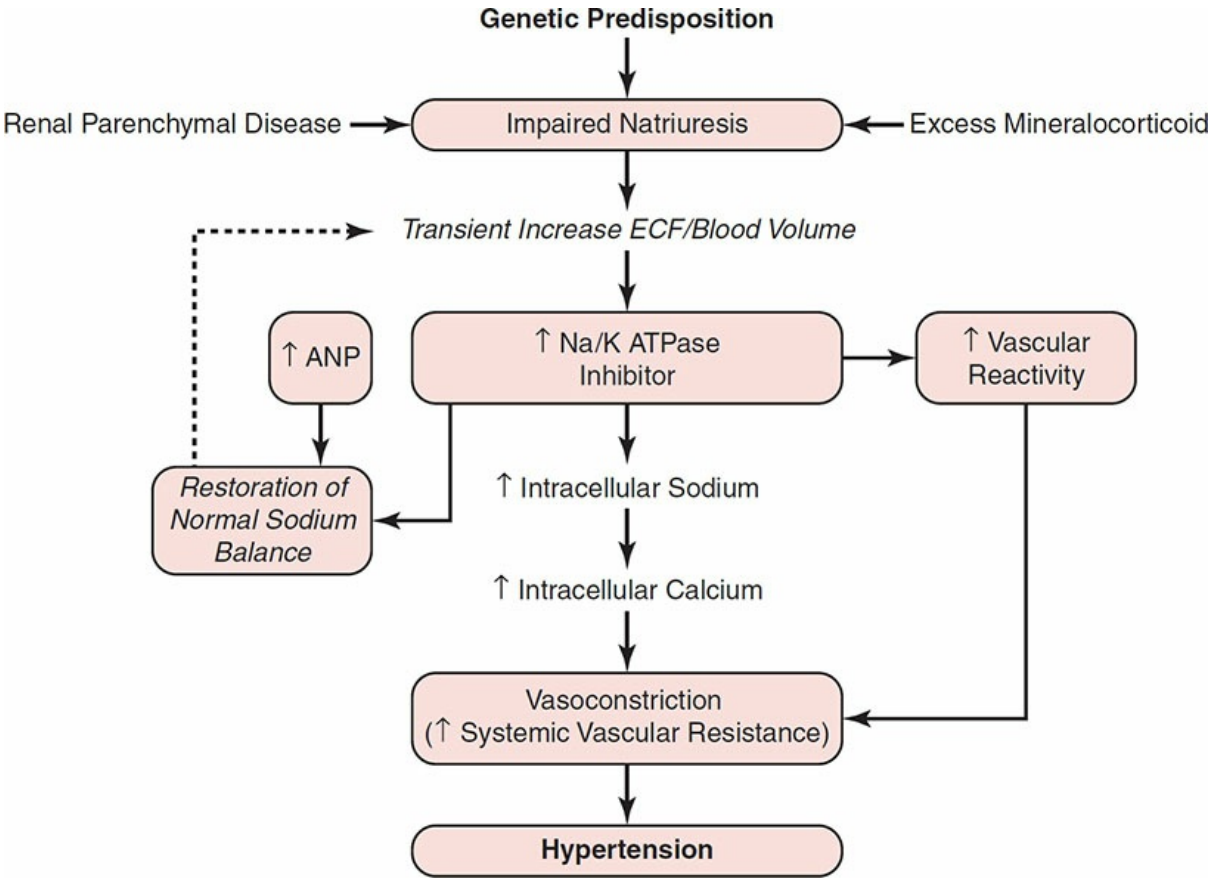

Figure 9-2 The Na/K ATPase inhibitor hypothesis. A defect in the inherent natriuretic capacity of the kidney is thought to be the fundamental abnormality that predisposes to the development of hypertension. The resulting tendency toward extracellular fluid (ECF) volume expansion leads to elaboration of two or more natriuretic hormones. Atrial natriuretic peptide (ANP) and a circulating inhibitor of the Na/K ATPase result in reduced renal tubular sodium reabsorption, thereby compensating for the underlying natriuretic defect and restoring sodium balance and ECF volume to normal. However, Na/K ATPase inhibition in vascular smooth muscle cells causes vasoconstriction because of increased intracellular calcium, resulting in systemic hypertension. ANP causes vasodilation, thereby attenuating the rise in blood pressure.

RENAL FUNCTION CURVES

Interactions of the renal perfusion pressure–sodium excretion mechanism and modulating neurohormonal factors normally operate very precisely to maintain sodium balance at a normal arterial pressure (92). The physiologic basis of the renal body fluid feedback mechanism for the regulation of arterial pressure is the direct effect of arterial pressure on output of water and sodium from the kidneys. Studies of the isolated, perfused kidney demonstrate the so-called pressure natriuresis and diuresis whereby an increase in perfusion pressure directly causes the renal output of sodium and water to increase (93,94). Figure 9-3 depicts a *renal*

function curve that shows the effect of perfusion pressure on urinary sodium excretion in the isolated perfused kidney. Urinary sodium output falls to zero when the arterial pressure falls to approximately 50 mm Hg. In contrast, the output of sodium increases sixfold to eightfold when the arterial pressure rises from the normal value of 100 to 200 mm Hg (92). This effect of arterial pressure on sodium excretion has been demonstrated in isolated, perfused kidneys and in intact animals. However, for the reasons discussed in the following, the upward slope of the renal function curve in the intact animal is much steeper. The horizontal line in Figure 9-3 represents the level of sodium intake at equilibrium when sodium intake and output are matched. When net intake and output of sodium are matched, the arterial pressure is determined by the point where the two plots intersect, which is known as the *equilibrium pressure point*. Computer model analysis of hypothetical renal function curves in the intact animal suggests that, if the renal function curve and the sodium intake remain unchanged, this is the unique perfusion pressure at which external sodium balance will be maintained (95). If the pressure rises above this level, the sodium output becomes greater than input and negative sodium balance occurs with eventual reduction in ECF volume and cardiac output to a level that returns the BP to the equilibrium point. In contrast, if the BP falls below the equilibrium point, the intake of sodium will be greater than the output and a positive sodium balance will occur until the increases in ECF volume, blood volume, and cardiac output are sufficient to return the pressure to the equilibrium point. Sodium balance can be maintained only at the 100 mm Hg equilibrium pressure point.

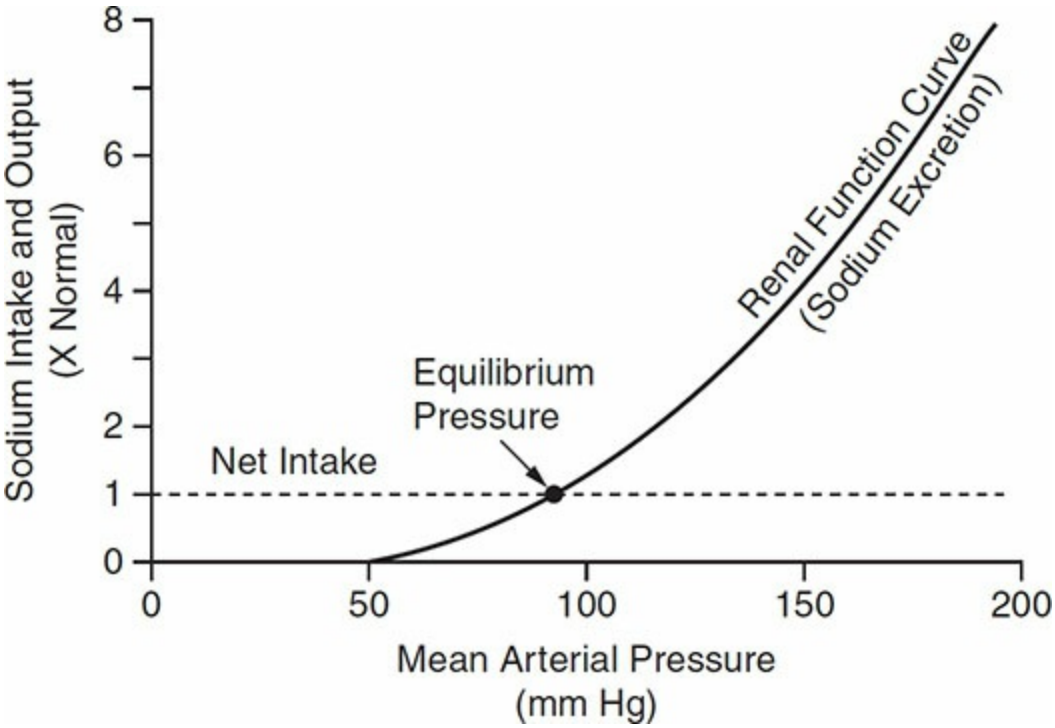

Figure 9-3 Renal function curves showing the effect of renal perfusion pressure on urinary sodium excretion in the isolated, perfused kidney. By using computer modeling, the corresponding sodium intake can be superimposed on the pressure natriuresis curve. An equilibrium pressure point is defined, which represents the unique level at which the arterial pressure will be regulated. It is apparent that a primary increase in systemic vascular resistance alone cannot lead to sustained increase in blood pressure (BP) because the pressure natriuresis mechanism would cause sodium excretion, decreased extracellular fluid (ECF) volume, and cardiac output, thereby returning the BP to the equilibrium point. Likewise, a primary decrease in systemic vascular resistance would not lead to sustained hypotension because reduced natriuresis, sodium retention, increased ECF volume, and cardiac output would once again return BP to the equilibrium point. (From Guyton AC. Renal function curve: a key to understanding the pathogenesis of hypertension. *Hypertension*. 1987;10:1, with permission from Wolters Kluwer Health, Inc.)

If this model, illustrated in Figure 9-3, is correct, then it is apparent that a primary increase in cardiac output or systemic vascular resistance cannot result in a sustained increase in BP because a normally functioning feedback mechanism would result in natriuresis and diuresis, thereby returning the BP to normal. Thus, a primary increase in systemic vascular resistance would be accompanied by an equal and opposite decrease in cardiac output with return of the BP to normal. This return of the pressure to the equilibrium point illustrates the infinite gain characteristic of the renal fluid–volume feedback mechanism. In this system, a change in the arterial pressure is the critical feedback stimulus that modifies the natriuretic response. In theory, if an initial decrease in BP results from a

decrease in cardiac output, for instance in the setting of congestive heart failure, then the compensatory salt and water retention would increase ECF volume but fail to normalize cardiac output and renal perfusion pressure. Thus, renal sodium and water retention would continue unopposed, resulting in massive fluid overload. This mechanism is consistent with the unifying hypothesis recently proposed to explain body fluid volume regulation in disorders characterized by low effective arterial blood volume (28,96). The implication of this renal fluid–volume feedback mechanism is that the finding of sustained hypertension must be a reflection of an underlying abnormality that caused a shift of the renal function curve to the right such that a higher BP is required to maintain sodium balance at any given level of sodium intake (97–99).

ROLE OF THE RENIN–ANGIOTENSIN SYSTEM IN REGULATION OF BLOOD PRESSURE

Unlike the isolated, perfused kidney or computer models, in vivo the position of the renal function curve can be shifted by various neural and endocrine factors. For example, changes in the activity of the RAAS can result in a shift of the curve and thus either magnify or blunt the basic relation between sodium and water excretion and BP (95,98). Different renal function curves can be produced in animals by varying sodium intake in stepwise increments while maintaining AII levels constant at various levels by using combinations of ACE inhibitor and AII infusion (Fig. 9-4) (97). With the AII level maintained above normal, there is a shift in the renal function curve to the right consistent with a blunting of the pressure-induced natriuretic response. In contrast, when AII is totally suppressed by an ACE inhibitor, the curve is shifted to the left, consistent with an exaggerated pressure natriuresis. The vertical line in Figure 9-4 represents a different type of renal function curve called a *salt-loading renal function curve*. It is obtained when sodium intake is varied in a stepwise fashion in animals with an intact RAAS (AII level allowed to vary in response to the sodium intake), which will modulate the intrinsic renal body fluid feedback mechanism. Thus, the renal function curve in the intact animal is much steeper than that seen in the isolated perfused kidney. In this salt-loading curve, BP at equilibrium for each level of sodium intake changes very little. Analysis of the superimposed renal function curves, with AII held constant at different levels, illustrates that the steepness of the curve in the intact animal may be owing to changes in the activity of the RAAS. A high sodium intake suppresses the RAAS and shifts the renal function

curve to the left, whereas a low sodium intake activates the RAAS and shifts the curve to the right. This modulation of the renal function curve by the RAAS is thought to result from the effect of AII on renal sodium and water reabsorption. AII directly enhances proximal tubular sodium reabsorption (74). In addition, AII has important renal hemodynamic effects that cause increased tubular sodium reabsorption independent of aldosterone (73). The predominant efferent vasoconstriction produced by AII causes a drop in peritubular capillary hydrostatic pressure, leading to enhanced tubular reabsorption of sodium and water (73,99). This dynamic interaction between the RAAS and renal fluid–volume feedback mechanism accounts for the observation that tremendous extremes of dietary sodium intake in normal individuals result in relatively little change in the systemic arterial pressure.

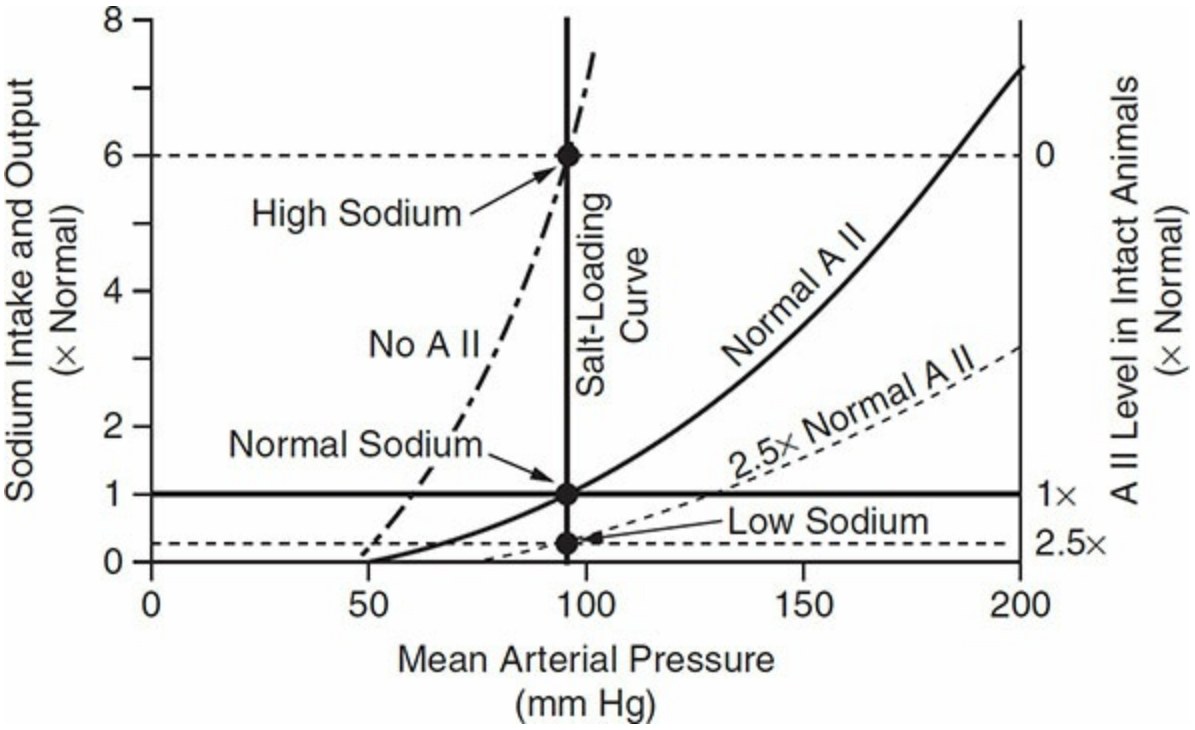

Figure 9-4 Modulation of renal function curves by the renin–angiotensin system. A salt-loading renal function curve is obtained when sodium intake is varied in stepwise increments in animals with an intact renin–angiotensin system. The curve is extremely steep such that over a wide range of dietary sodium intake, the blood pressure (BP) changes very little. Three separate renal function curves were produced by varying the sodium intake in a stepwise fashion in animals, with angiotensin II (AII) levels maintained constant in either the normal range, 2.5 times normal, or with AII absent. The curve obtained when AII production is suppressed by angiotensin-converting enzyme inhibitor is shifted to the left, consistent with an enhanced natriuretic response to any given level of perfusion pressure. In contrast, with an AII level at 2.5 times normal, the curve is shifted to the right, reflecting a blunting of the intrinsic renal

natriuretic response. Superimposition of these individual renal function curves reveals that the steepness of the salt-loading renal function curve in the intact animal is caused by changes in the natriuretic response to pressure, which are in turn mediated by changes in the activity of the renin–angiotensin system as a function of dietary sodium intake. AII is suppressed with high sodium intake, producing a shift of the renal function curve to the left so the salt load can be excreted at normal BP. In contrast, AII production is enhanced with dietary sodium restriction, leading to a shift of the curve to the right such that the kidney is more avid for sodium such that a normal level of BP is still maintained. (From Guyton AC. Renal function curve: a key to understanding hypertension. *Hypertension*. 1987;10:1, with permission from Wolters Kluwer Health, Inc.)

AUTOREGULATION LEADS TO INCREASED SYSTEMIC VASCULAR RESISTANCE

The ability to maintain normal BP over a wide range of dietary sodium intake is present only if both the RAA system and the kidney function normally. Aberrations in either the RAA system or the renal fluid–volume mechanism can lead to a significant increase in BP when the sodium intake is increased. Guyton's hypothesis holds that there are two basic ways in which the pressure equilibrium set point can be increased with resulting hypertension. A shift of the renal function curve to the right along the pressure axis owing to either intrinsic renal abnormalities or overactivity of the RAA system can cause hypertension. Alternatively, an increase in the sodium intake without a compensatory leftward shift of the renal function curve also can cause hypertension.

In the setting of an underlying decrease in the inherent natriuretic capacity, the renal fluid–volume feedback mechanism should cause progressive sodium and water retention until the increases in ECF volume, blood volume, and cardiac output are sufficient to raise the BP to the equilibrium pressure point. Sodium and water balance is restored at the equilibrium point; however, this is accomplished at the expense of systemic hypertension (Fig. 9-5). Thus, when the inherent natriuretic capacity is reduced, Guyton's concept holds that an increase in arterial pressure is an essential protective mechanism to restore sodium balance and avert disaster (21–25,94).

Theoretically, hypertension caused by a rightward shift of the renal function curve should be mediated by an increased cardiac output in response to sodium and water retention. However, in animal models and humans with essential hypertension, even though the initiating mechanism for hypertension is sodium retention with increased ECF volume and

cardiac output, ultimately an increase in the peripheral vascular resistance perpetuates the hypertension, whereas the cardiac output and ECF volume return to normal. According to Guyton's hypothesis, this transition from high cardiac output hypertension to the high systemic vascular resistance type of hypertension is explained by the process of autoregulation of blood flow in the systemic circulation (23–25). *Autoregulation* is a local tissue phenomenon that adjusts local blood flow when it becomes too high or low. Acutely, autoregulation may result from local changes in vascular muscle tone; however, structural changes in the resistance vessels develop in the chronic phase (18). In theory, when the cardiac output increases as a result of ECF volume expansion, autoregulatory vasoconstriction in all vascular beds eventually returns the cardiac output to normal. Hypertension persists, however, because the fall in cardiac output is accompanied by an equal and opposite increase in systemic vascular resistance. Given the persistent hypertension, sodium balance can still be maintained by the renal fluid–volume pressure natriuresis mechanism. Figure 9-5 summarizes the pathophysiologic sequence whereby the various disorders that cause an initial defect in natriuretic capacity (shift of renal function curve to the right) eventually lead to sustained hypertension caused by increased systemic vascular resistance in the absence of clinically evident increases in ECF volume, blood volume, or cardiac output.

RENAL FUNCTION CURVES IN SALT-SENSITIVE AND -RESISTANT ESSENTIAL HYPERTENSION

An impairment of the intrinsic natriuretic capacity of the kidney is easy to conceptualize in the setting of the mendelian forms of hypertension, renal artery stenosis, primary renal parenchymal disease, or mineralocorticoid-induced hypertension. However, in the early stages of human essential hypertension, no specific renal histologic abnormality can be identified. At face value, this observation suggests that renal function is entirely normal until damage (nephrosclerosis) secondary to hypertension supervenes. However, if either the Guyton hypothesis or the Na/K ATPase inhibitor hypothesis is correct, it is clear that with sustained hypertension, regardless of etiology, there must be a rightward shift of the renal function curve such that sodium balance is maintained at a hypertensive level.

Salt balance is maintained at a normal BP in the normal individual. Moreover, the slope of the renal function curve is very steep, such that even with dietary salt loading the BP remains near normal (Fig. 9-6). Two

subsets of essential hypertension (salt-sensitive and salt-resistant) have been identified based on the response of BP to increases in dietary sodium intake (100,101). Approximately 60% of subjects with essential hypertension have greater than a 10% increase in BP when given a high-sodium diet (>200 mmol/day) and are defined as salt-sensitive, whereas 40% are salt-resistant. PRA is low in salt-sensitive patients and usually normal or high in salt-resistant patients. These hypertensive subtypes probably represent differences in the adaptation to a sodium load. The salt-loading renal function curve in *salt-resistant hypertension* is shifted to the right but it remains parallel to the curve for normotensive individuals (Fig. 9-6). Thus, salt balance is maintained on a normal sodium intake, but at a higher BP set point. However, because the renal function curve is steep, the BP does not increase further with dietary salt loading. In contrast with *salt-sensitive hypertension*, the rightward shift of the curve is accompanied by a depression of the slope. Thus, not only is the BP set point on a normal sodium diet elevated, but also the BP increases in response to dietary salt loading (Fig. 9-6). Response to strict dietary sodium restriction also differs in the two subtypes. Hypertension responds to reduced sodium intake in salt-sensitive hypertension but not in salt-resistant hypertension.

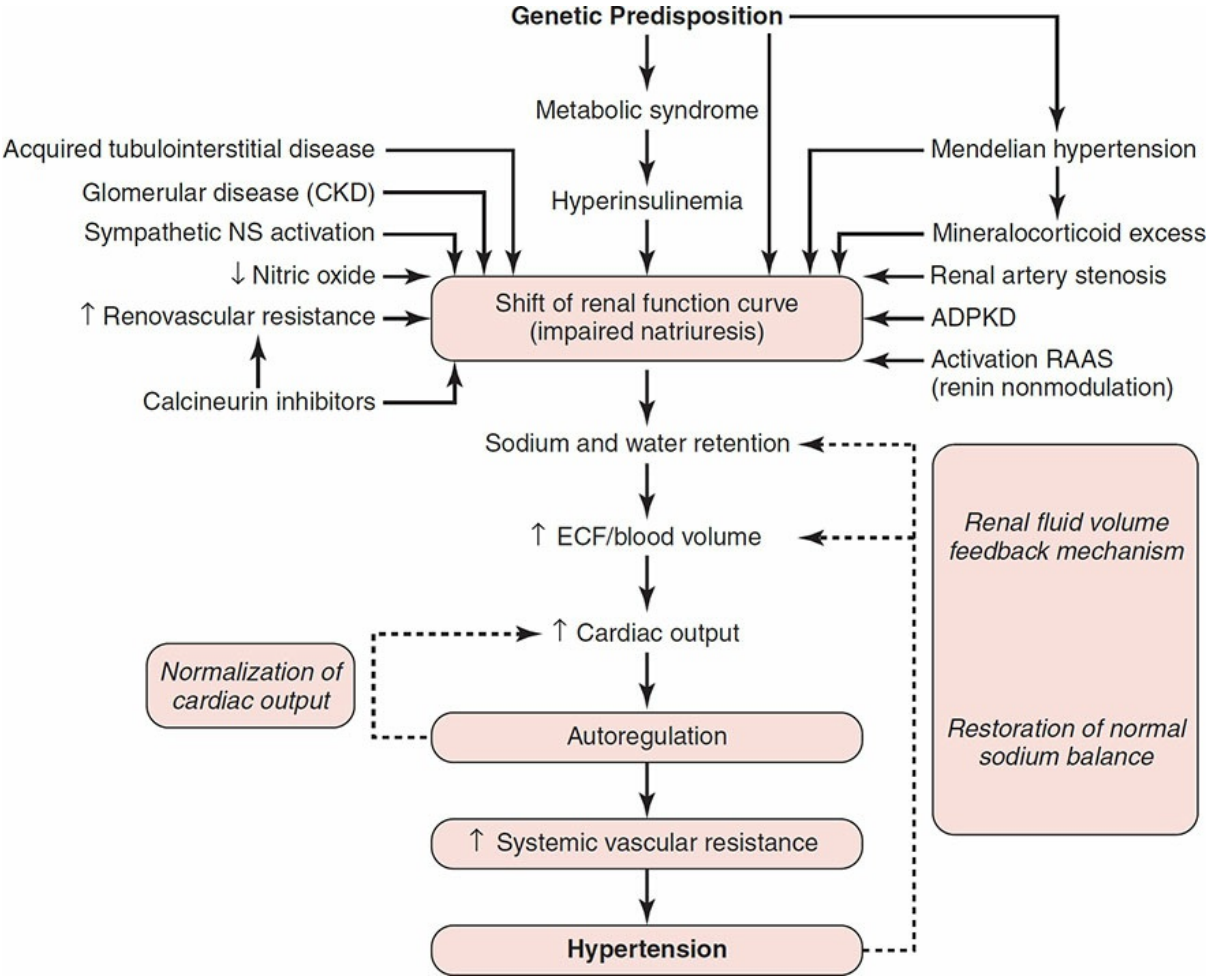

Figure 9-5 The Guyton hypothesis. A defect in the inherent natriuretic capacity of the kidney is thought to be the fundamental abnormality that predisposes to the development of all forms of hypertension. A variety of disorders can lead to a shift in the intrinsic renal function curve with reduced natriuretic capacity. Causes of impaired natriuresis include a genetic predisposition to essential hypertension, primary renal parenchymal disease caused by diabetic nephropathy or other primary glomerular disease with nephron loss, acquired tubulointerstitial disease, inherited or acquired glucose intolerance with hyperinsulinemia, mineralocorticoid excess states, mendelian forms of hypertension that lead to enhanced tubular sodium reabsorption, failure to downregulate the renin–angiotensin in response to volume expansion (renin nonmodulation), renal artery stenosis, activation of sympathetic nervous system (catecholamines or renal nerves), and decreased renal nitric oxide. A rightward shift of the renal function curve with impaired natriuresis is thought to be the fundamental abnormality that underlies all causes of hypertension. Initially, sodium and water retention lead to increases in extracellular fluid (ECF) volume and cardiac output. In the long term, circulatory autoregulation restores cardiac output to normal. However, autoregulation also leads to a sustained increase in systemic vascular resistance and systemic hypertension. Via pressure-induced natriuresis, the renal fluid–volume feedback mechanism, returns sodium balance and ECF volume to normal but at the expense of sustained hypertension. Guyton's hypothesis explains the paradox whereby a primary disorder that involves enhanced renal sodium reabsorption ultimately results in

hypertension with elevated systemic vascular resistance in the absence of a detectable increase in ECF volume.

Experimental evidence suggests that disorders associated with increased renal vascular resistance, such as one-kidney Goldblatt hypertension, tend to induce salt-resistant hypertension. In contrast, salt sensitivity with rightward shift of the curve accompanied by a depression in slope occurs in conditions characterized by increased sodium reabsorption by the renal tubules. This phenomenon occurs in DOC-salt hypertension in the rat, in patients with primary hyperaldosteronism, in the various mendelian forms of hypertension with enhanced tubular sodium reabsorption, in the setting of reduced renal mass or CKD, and in conditions characterized by blunting of the normal negative feedback of the RAAS. In these instances, as sodium intake increases, an incremental rise in BP is required to overcome excessive sodium reabsorption and maintain normal sodium balance (100).

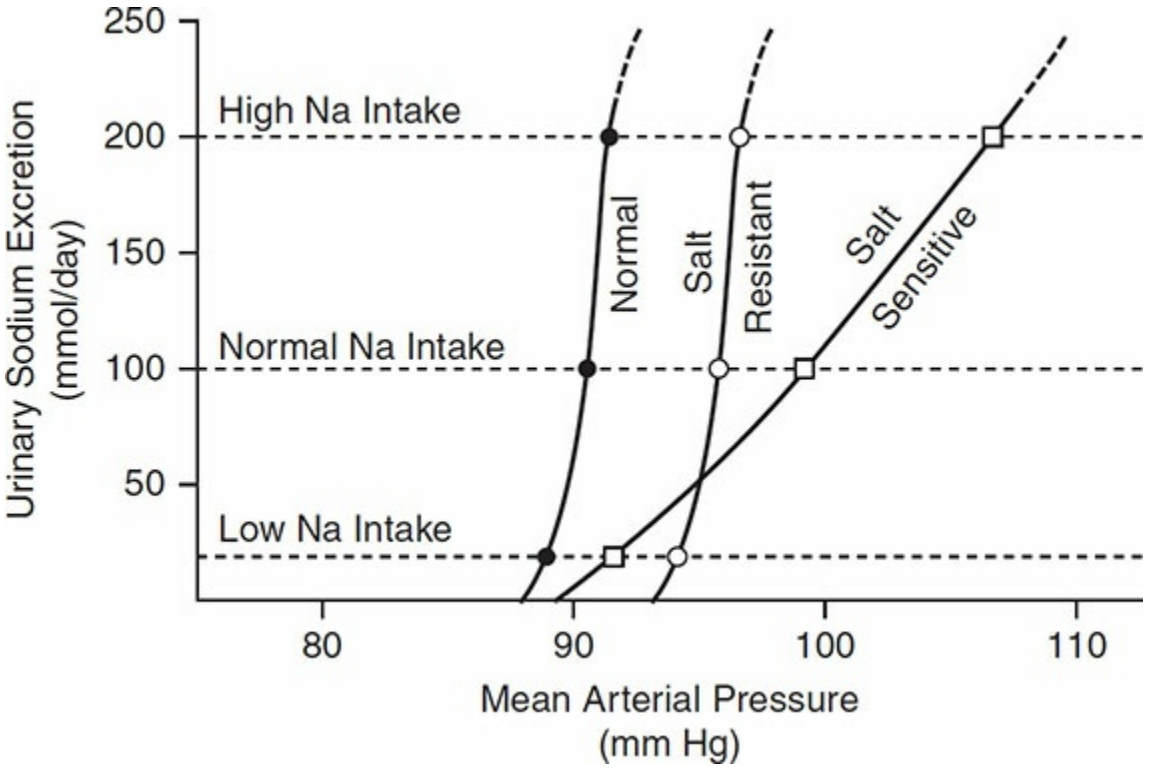

Figure 9-6 Schematic renal function curves in human essential hypertension. In the normal individual, salt balance is maintained at a normal blood pressure (BP). Moreover, the slope of the normal renal function curve is very steep, such that the BP remains near normal even with dietary sodium loading. The salt-loading renal function curve in *salt-resistant hypertension* is shifted to the right, but remains parallel to the curve for normotensive individuals. Thus, salt balance is maintained during normal sodium intake, but at a higher BP set point. However, because the renal function curve is steep, the BP does not increase during dietary salt loading. In contrast, in *salt-*

sensitive hypertension, the rightward shift of the curve is accompanied by a depression of the slope. Thus, not only is the BP set point on a normal sodium diet elevated but also the BP increases in response to dietary salt loading. The response to strict dietary sodium intake differs in the two subtypes of essential hypertension as well. Hypertension responds to lowered sodium intake in salt-sensitive hypertension, but not in salt-resistant hypertension.

The precise nature of the defect responsible for the altered pressure natriuresis mechanism in human essential hypertension is unknown. Theoretically, any abnormality that increases renal vascular resistance, reduces renal mass, decreases glomerular basement membrane filtration coefficient, and increases tubular sodium reabsorption (angiotensin, α-adrenergic stimulation, deficient renal NO, aldosterone or other mineralocorticoids, and alterations in net peritubular Starling forces) could impair renal natriuretic capacity and lead to hypertension (23). Changes in renal vascular resistance have been clearly documented in human essential hypertension, especially with advanced nephrosclerosis. On the other hand, the mechanism of increased tubular sodium reabsorption in salt-sensitive patients may relate to abnormalities of the sympathetic nervous system (100). Salt-sensitive patients display an abnormal relation between sodium intake and plasma NE levels. Although plasma NE levels are suppressed by salt loading in normal individuals and salt-resistant patients, they tend to increase in salt-sensitive patients. It has been postulated that increased sympathetic activity and reduced renal sodium excretion in salt-sensitive patients may be related to a defect in sodium-coupled cellular calcium transport. In this regard, CCBs have been shown to have a natriuretic effect and to normalize the derangements in the renal function curve in salt-sensitive hypertension in blacks (79,100).

THE DOMINANT ROLE OF PERFUSION RENAL PERFUSION PRESSURE IN MINERALOCORTICOID ESCAPE

To substantiate the role of direct pressure-induced natriuresis in the regulation of sodium balance in mineralocorticoid hypertension, Hall et al. compared the systemic BP and natriuretic effect of aldosterone infusion in a dog model in which the renal perfusion pressure was either allowed to increase or mechanically servocontrolled to maintain perfusion pressure at normal levels (Fig. 9-7) (101). In the intact animal, continuous aldosterone infusion caused a transient period of sodium and water retention with a mild increase in BP. However, the sodium retention lasted only a few days and was followed by an escape from the sodium-retaining effects of

aldosterone and restoration of normal sodium balance. In contrast, when the renal perfusion pressure was mechanically servocontrolled at a normal level during aldosterone infusion, there was no escape from aldosterone leading to relentless increase in sodium and water retention accompanied by severe hypertension, edema, ascites, and congestive heart failure. When the servocontrol device was removed and the perfusion pressure was allowed to rise to the systemic level, a prompt natriuresis and diuresis ensued with restoration of sodium balance and a fall in BP. Similar observations have been made in studies of hypertension produced by AII (102) or vasopressin infusion (103). These observations highlight the pivotal role of BP in the regulation of renal sodium and water excretion. It seems that the natriuretic factors proposed in the Na/K ATPase inhibitor hypothesis are not sufficient to offset the antinatriuretic action of mineralocorticoids in the absence of an accompanying increase in renal perfusion pressure.

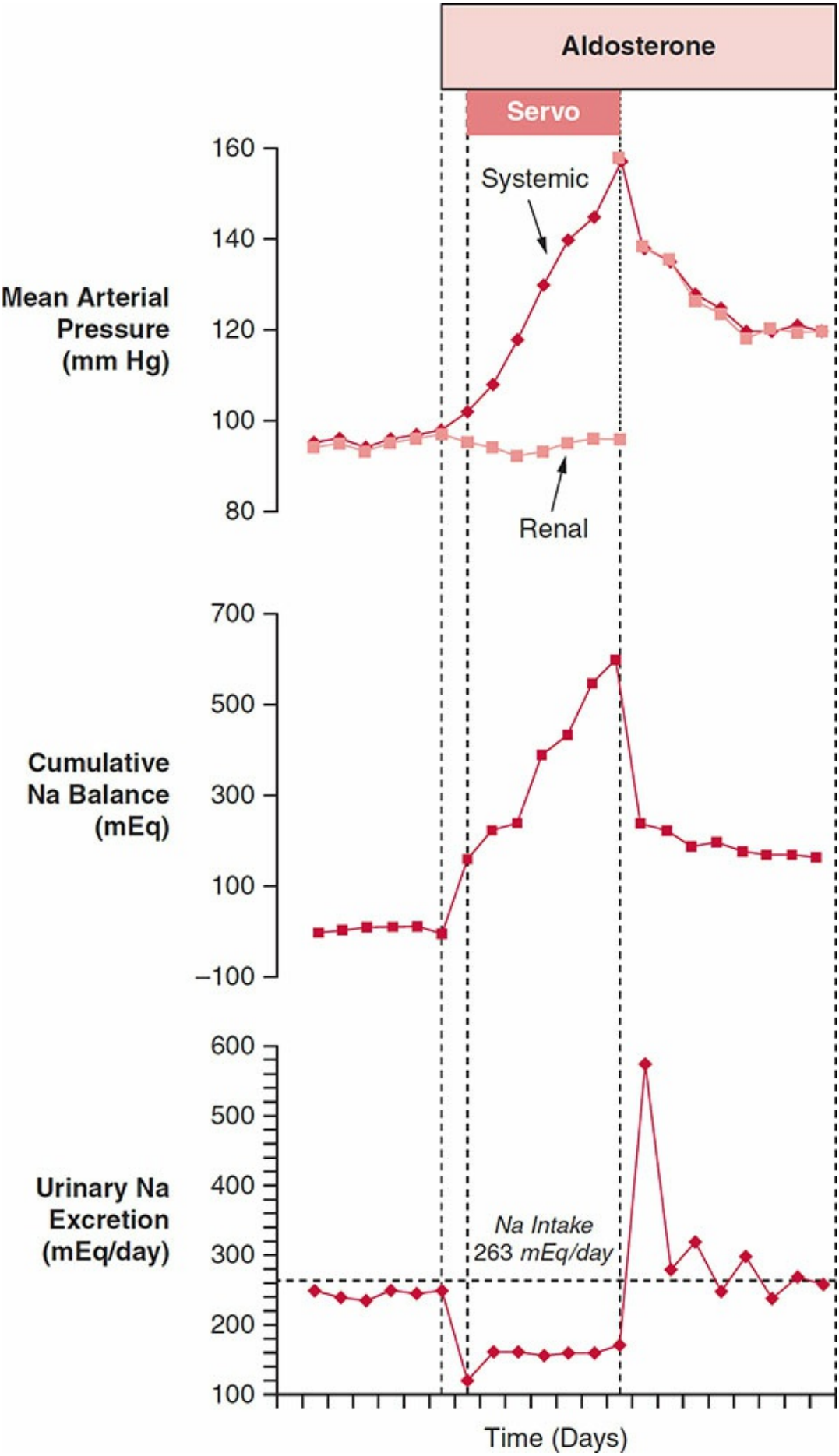
Aldosterone
Servo
Systemic
Renal
Mean Arterial Pressure (mm Hg)
160
140
120
100
80
Cumulative Na Balance (mEq)
700
500
300
100
−100
Urinary Na Excretion (mEq/day)
600
500
400
300
200
100
Na Intake
263 mEq/day
Time (Days)

Figure 9-7 The dominant role of renal perfusion pressure in the mineralocorticoid escape phenomenon. In the intact animal, treatment with aldosterone and ingestion of a high-salt diet leads to sodium retention and volume expansion for only a few days, after which a spontaneous natriuresis and diuresis occurs that returns extracellular fluid volume toward normal—the so-called mineralocorticoid escape phenomenon. This figure demonstrates the pivotal role of elevated renal perfusion pressure in the escape of the kidney from the sodium-retaining effects of aldosterone. Dogs were placed on a 14-day infusion of aldosterone in conjunction with a high-salt diet (263 mEq sodium per day). However, during the first 7 days the renal perfusion pressure was servocontrolled at normal levels by use of a suprarenal aortic clamp. Despite the development of systemic hypertension, when the renal perfusion pressure is normal, urinary sodium excretion remains less than intake and aldosterone escape fails to occur. The net result is inexorable salt and water retention, resulting in pulmonary edema and marked systemic hypertension. However, after day 7, when the clamp is removed such that renal perfusion pressure matches the elevated systemic pressure, there is an immediate natriuresis and diuresis (escape), leading to a return of sodium balance toward normal despite continued aldosterone administration. The systemic blood pressure improves but remains well above the baseline. Servo, servocontrolled renal perfusion pressure to normal level with supra-aortic cuff. (From Hall JE, Granger JP, Smith MJ, et al. Role of renal hemodynamics and arterial pressure in aldosterone "escape." *Hypertension*. 1984; (2, pt 2):I183, with permission from Wolters Kluwer Health, Inc.)

MOLECULAR MECHANISMS OF PRESSURE NATRIURESIS

The phenomenon of pressure natriuresis may be multifactorial and may differ in mechanism depending on whether the increase in arterial pressure is acute (minutes) or chronic (hours to days). In the setting of an acute increase in arterial pressure achieved by cross clamping the infrarenal aorta, pressure natriuresis appears to be the result of inhibition of NaCl absorption in the PT as the result of endocytosis of sodium transporters in the apical (NHE-3) and basolateral (Na^+/K^+ ATPase) membranes of the PT (104). In contrast to the findings with acute hypertension, long-term pressure natriuresis is dependent on inhibition of sodium transport in more distal nephron segments. As discussed, in animal models of primary hyperaldosteronism, within a few days the kidneys escape from the sodium-retaining effects of aldosterone via a pressure-mediated natriuresis that acts to return sodium balance and ECF volume to normal. Knepper et al. studied the molecular mechanism of pressure natriuresis in a rat model of primary hyperaldosteronism by using a targeted-proteomics approach (105). They screened rat kidney protein homogenates with rabbit polyclonal antibodies specific for each of the major sodium transporters expressed along the nephron to determine whether escape from

aldosterone-mediated sodium retention is associated with decreased abundance of one or more of the transporters. The analysis revealed that the abundance of the thiazide-sensitive NCC was profoundly and selectively decreased during aldosterone escape. The decrease in NCC abundance occurred with a time course of onset that paralleled the increase in renal NaCl excretion associated with the escape process and NCC abundance fell to 17% to 25% of baseline levels. None of the other apical solute-coupled Na transporters (NHE-3, NaPi-2, or the Na/K/2Cl cotransporter) displayed decreased abundance, nor were the total abundance of the three ENaC subunits significantly altered. Immunohistochemical staining confirmed a substantial decrease in NCC labeling in the DCTs of aldosterone-escape rats. Ribonuclease protection assay showed that the decrease in NCC protein abundance was not associated with a significant change in mRNA abundance for NCC, which implies that the decrease in NCC expression in aldosterone escape occurs because of a posttranscriptional mechanism. Thus, downregulation of the thiazide-sensitive NCC of the DCT appears to be the chief molecular target for the regulatory processes responsible for pressure-induced natriuresis in mineralocorticoid escape. Taken together, these observations highlight the pivotal role of BP in the regulation of renal sodium and water excretion.

Essential Hypertension and Benign Nephrosclerosis

The kidney is usually histologically normal in the early stages of essential hypertension. However, with time, there is a gradual loss of nephron mass so that a contracted, granular kidney is found with long-standing benign hypertension. This progressive reduction in renal size is caused by diffuse cortical atrophy and fibrosis owing to hyaline arteriosclerosis, the severity of which is proportional to the duration of the hypertension (106). In benign nephrosclerosis, the afferent arterioles demonstrate hyaline arteriosclerosis with subintimal deposition of a homogenous eosinophilic material (Fig. 9-8A). The interlobular arteries exhibit fibroelastic hyperplasia, which consists of concentric rings of reduplication of the internal elastic lamina (Fig. 9-8B). There is patchy ischemic atrophy of glomeruli; although some glomeruli are normal, others are globally sclerotic. Atrophic tubules filled with eosinophilic material are seen in the areas of glomerular ischemia (Fig. 9-8A).

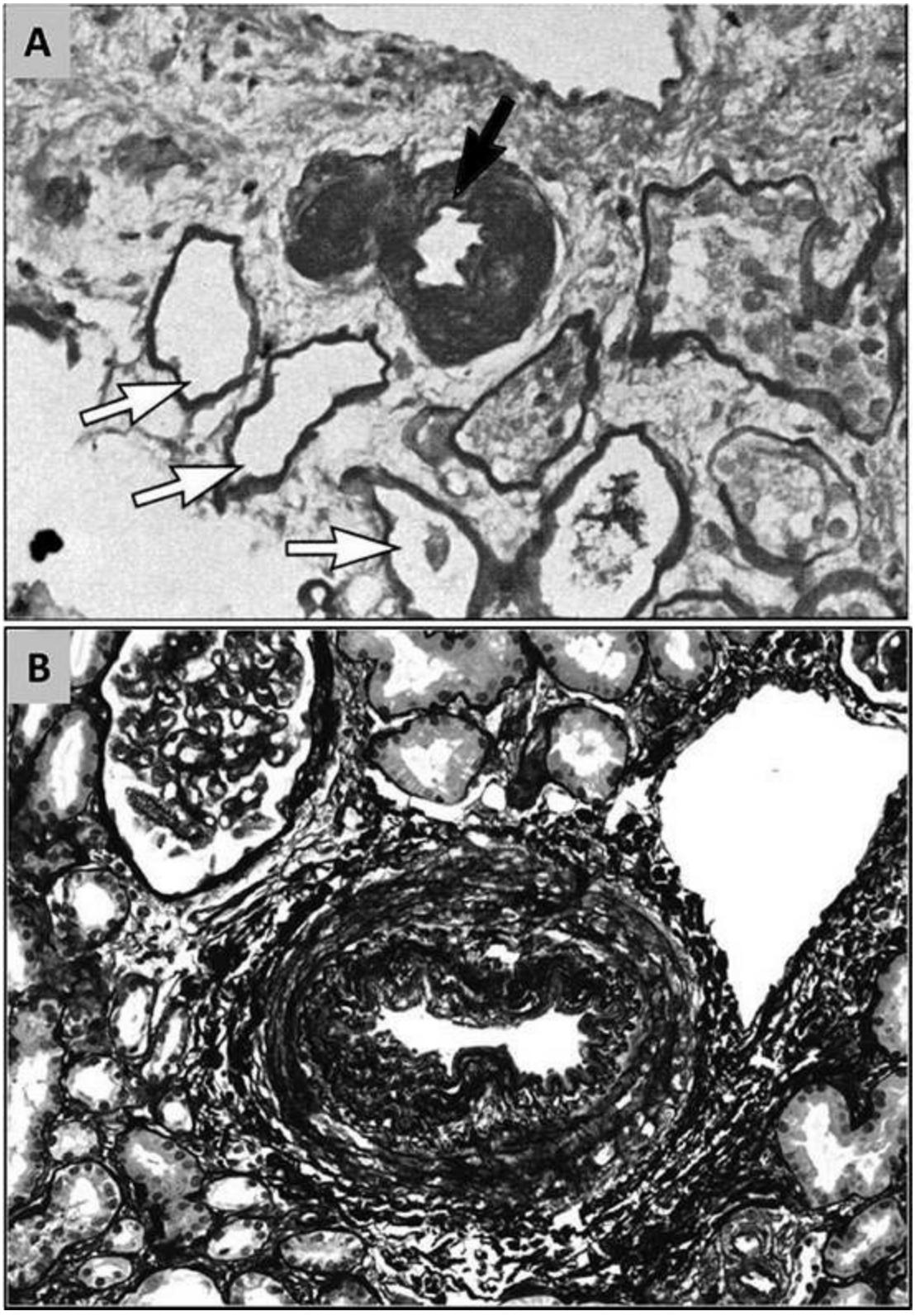

Figure 9-8 (**A**) Hyaline arteriolar nephrosclerosis in benign hypertension. In benign arteriolar nephrosclerosis caused by benign hypertension, the characteristic lesion is hyaline arteriolosclerosis with expansion of the intima of afferent arterioles by an eosinophilic amorphous hyaline material which stains a pale pink color on periodic acid-Schiff (PAS) stain (*black arrow*). There is patchy ischemic atrophy of the glomeruli (*data not shown*). Some glomeruli are normal, whereas others are completely

hyalinized. Atrophic tubules (*white arrows*), sometimes filled with amorphous material, may be seen in the area of sclerotic glomeruli. The severity of tubular atrophy, interstitial fibrosis, and glomerulosclerosis is proportional to the extent of vascular involvement with hyaline arteriosclerosis. (**B**) Fibroelastic hyperplasia of interlobular arteries in benign nephrosclerosis. In benign hypertension, the interlobular arteries (cortical radial arteries) are thickened as a result of extensive reduplication of the internal elastic lamina. Reduplicated bands of elastic lamina can be visualized using elastin stain or methenamine silver stain. These arterial changes in benign hypertension stand in marked contrast to the proliferation of myointimal fibroblasts typical of malignant nephrosclerosis (see Fig. 9-9). (From Sherry Werner-Abboud, MD; University of Texas Health Sciences Center at San Antonio, with permission.)

BENIGN NEPHROSCLEROSIS AS A CAUSE OF END-STAGE RENAL DISEASE

Despite the presence of these renal histologic abnormalities, even with long-standing benign hypertension, the majority of patients with essential hypertension never develop clinically significant renal insufficiency. Benign essential hypertension tends to cause much less damage to the kidney than to other target organs such as the heart and brain. Indeed, the relationship between benign essential hypertension and ESRD remains circumstantial despite the fact that these syndromes have long been associated in the medical literature. The widely held notion that benign hypertension with benign nephrosclerosis is a common cause of ESRD is difficult to support. Recent reviews have suggested that the number of patients reaching ESRD attributable to benign nephrosclerosis may have been significantly overestimated (107–109). Based on completion of the Health Care Financing Administration (HCFA) 2728 form, practicing nephrologists attribute essential hypertension as the cause of ESRD in 37% of patients initiating Medicare-supported renal replacement therapy (110). Unfortunately, the cause of ESRD more often is based on clinical parameters rather than histologic diagnosis. Two different clinical guidelines have been proposed for the phenotypic identification of hypertensive nephrosclerosis. One guideline suggested that the clinical diagnosis of hypertensive nephrosclerosis includes a family history of hypertension, and the presence of left ventricular hypertrophy, proteinuria <0.5 g/day, and hypertension preceding the onset of renal dysfunction (109). The African American Study of Kidney Disease (AASK) study investigators required a urine protein-to-creatinine ratio <2.0 and no evidence of underlying renal disease for the clinical diagnosis of hypertensive nephrosclerosis (111). Phenotyping of 100 randomly selected

patients with a diagnosis of hypertensive nephrosclerosis reported on the HCFA 2728 form revealed that only 4% of the patients met either clinical criterion for diagnosis (110). Analysis of the data for black patients revealed that only 28/91 patients met the AASK criteria for diagnosis of hypertensive nephrosclerosis (110). These data indicated that benign hypertensive nephrosclerosis is a far less common cause of ESRD than commonly assumed and suggest that many patients with presumed hypertensive nephrosclerosis may actually have other undetected causes of CKD. Indeed, the biopsy study demonstrated extensive global sclerosis, in excess of the patients' age suggesting that the kidney disease may have preceded the hypertension (112). Overall, the development of significant renal dysfunction appears to be very rare in uncomplicated essential hypertension. Estimates in white populations have suggested that the relative risk of developing renal failure in essential hypertension is on the order of 1 in 6,000 cases (111,113). Moreover, serum creatinine levels infrequently increase in patients with long-standing mild to moderate hypertension. An analysis of the data from three recent large clinical trials in patients with essential hypertension revealed that <1% of 10,000 patients developed advanced renal failure during 4 to 6 years of follow-up (114,115). A very low incidence of clinically significant deterioration of renal function was also noted in the Hypertension Detection and Follow-Up Program (HDFP) (116).

Autopsy studies conducted in the preantihypertensive era have documented that benign nephrosclerosis is an uncommon cause of ESRD disease. Among 150 hypertensive patients with ESRD, only one was found to have benign nephrosclerosis as the sole underlying etiology (117). Over the years, some authors have maintained that patients with hypertension and renal impairment, in whom renal artery stenosis (ischemic nephropathy) and malignant hypertension have been excluded, most likely have underlying primary renal parenchymal disease rather than benign nephrosclerosis (118).

Patients classified as having hypertensive ESRD typically present with advanced disease, making the processes that initiated the renal disease difficult to discern. It has been proposed that many patients misclassified with ESRD secondary to benign nephrosclerosis actually have primary renal parenchymal disease (such as immunoglobulin A [IgA] nephropathy), unrecognized renal artery stenosis with ischemic nephropathy, unrecognized episodes of malignant hypertension, occult renal cholesterol embolic disease, or primary renal microvascular disease (108,109,113).

Hemodynamic studies in essential hypertension demonstrate a near-normal GFR despite a significant reduction in renal blood flow, consistent with an increased filtration fraction. Genetic models of essential hypertension in the rat have shown that these alterations in renal hemodynamics arise through an increase in resistance of both the afferent and efferent arterioles, so that glomerular capillary hydraulic pressure is maintained at a normal level (119). In humans with benign essential hypertension, there also appears to be a balanced increase in afferent and efferent resistances, thereby shielding the kidney from the high systemic pressure, while enabling the maintenance of near-normal GFR. The relative rarity of significant renal impairment in patients with essential hypertension (nonmalignant) is consistent with these hemodynamic observations. In contrast, in animal models of diabetic nephropathy (120) and renal ablation (121), afferent arteriolar vasodilatation occurs such that an increase in arterial pressure is transmitted to the glomeruli. As already discussed, the resulting glomerular capillary hypertension in this setting may be a critical factor in the progression of CKD in patients with diabetic nephropathy or another primary renal parenchymal disease.

HYPERTENSION AND END-STAGE RENAL DISEASE IN AFRICAN AMERICANS

The overall rate of ESRD is significantly higher in blacks than in whites (122). It had been suggested that the higher incidence of ESRD in blacks compared to whites may be the consequence of a higher risk of progressive hypertensive nephrosclerosis among African Americans. Epidemiologic studies suggested that essential hypertension occurs more frequently in blacks and is associated with more severe cardiovascular end-organ damage for any given level of BP (123). In earlier angiographic studies of patients with mild to moderate essential hypertension and normal renal function, blacks tended to have more severe angiographic evidence of nephrosclerosis than whites (124). This notion has been subsequently confirmed in the biopsy study of AASK where study participants were found to have extensive global sclerosis that far exceeded expected based on the patients' age suggesting that the kidney disease in this patient population differs from that of hypertensive nephrosclerosis (112).

The discovery of a link between ApoL1 genetic variants and progressive kidney disease in African Americans has further supported the notion that "hypertensive nephrosclerosis" in African Americans is a different disease and that the manifestation of progressive kidney disease

is at least partially due to genetic predisposition (17). ApoL1 is a minor apolipoprotein component of high-density lipoprotein cholesterol. It is expressed in the kidney (125) and in other tissues including the lung, vasculature, and placenta (126). Although the exact function of ApoL1 remains unknown, ApoL1 circulating in plasma has the ability to kill the trypanosome *Trypanosoma brucei* that causes sleeping sickness (127). The genetic variants associated with kidney disease are more prevalent in western compared to northeastern African populations and absent in Ethiopia (128). The frequency of risk alleles in African Americans, many of whom are descendants of people of West African nations, is high (>30%) (17). This form of primary glomerulosclerosis and arteriolar nephrosclerosis with resultant glomerular ischemia is likely the cause of the hypertension and vascular disease risk factors leading some to call for a change to the name of the disease (129). It is important to note, that while the prevalence of the risk alleles is high in African Americans with nondiabetic kidney diseases, not all individuals with these alleles develop kidney disease (130) suggesting that other factors may still play a role.

In this regard, there are several plausible explanations for the high frequency with which “hypertensive nephrosclerosis” is reported as a cause of ESRD in the black population. One possibility is that recurrent bouts of unrecognized or inadequately treated malignant hypertension are the actual cause of the increase in ESRD, owing to hypertension among blacks. The incidence of malignant hypertension is higher in blacks than in whites. In the few older studies detailing the pathologic findings in blacks with ESRD caused by hypertension, the characteristic findings have been those of malignant nephrosclerosis, namely, musculomucoid intimal hyperplasia of the interlobular arteries and accelerated glomerular obsolescence, rather than benign arteriolar nephrosclerosis (109,131). In this regard, a study of 100 patients admitted to an inner-city hospital with a diagnosis of hypertensive emergency showed that two-thirds had malignant hypertension on the basis of funduscopic findings (132). These patients were predominantly young, male, black, or Hispanic individuals of lower socioeconomic status. Over 93% of these patients had been previously diagnosed as hypertensive and most reported to have received prior pharmacologic treatment for hypertension. However, no source of regular health care could be documented in 60% of cases. More than 50% were noted to have stopped their antihypertensive medications more than 30 days before admission and only 24% had taken any medication on the day of admission. If the overrepresentation of young blacks with ESRD is at least in part owing to undiagnosed or inadequately treated malignant

hypertension, this would have tremendous public health implications because malignant hypertension is clearly preventable, and even significant renal dysfunction is potentially reversible with early and aggressive antihypertensive therapy.

Additionally, environmental dietary factors may be at play. Tobian has postulated that the low-potassium diet characteristically consumed by blacks in the United States (30 vs. 65 mmol/day in the general population) accelerates the intimal thickening of the renal vasculature that occurs because of hypertensive damage and might contribute to their increased risk of progressive renal disease with benign hypertension. He has proposed that this may account for the increased risk of progressive renal insufficiency among hypertensive blacks (133).

However, perhaps the most relevant factor is salt sensitivity. Compared to whites, blacks tend to have a more expanded intravascular volume, lower PRA, reduced natriuretic response to a sodium load, and better antihypertensive responses to diuretics and CCB than ACE inhibitors or β-blockers. Substantial renal hemodynamic differences between black and white patients with supposed essential hypertension have been described (75,100). For instance, black hypertensive patients have greater reduction in renal blood flow and higher renal vascular resistance than white patients (100). In addition, black hypertensive patients are more likely to be salt-sensitive than are white hypertensives such that an increase in sodium intake leads to an increase in BP (75). In a study of 17 black patients and 9 white patients with hypertension, 11 blacks were found to be salt-sensitive, whereas all the whites were salt-resistant (75). Renal hemodynamics were measured during a low-sodium diet (20 mmol/day for 9 days) and a high-salt diet (200 mmol/day for 14 days). During the low-sodium diet period, salt-sensitive and salt-resistant patients had similar MAP, GFR, effective renal plasma flow (ERPF), and filtration fraction. During the high-salt diet period, GFR did not change in either group; ERPF increased in salt-resistant patients but decreased in salt-sensitive patients; filtration fraction decreased in salt-resistant patients but increased in salt-sensitive patients; glomerular pressure decreased in salt-resistant patients but increased in salt-sensitive patients. In the salt-sensitive patients, the stable GFR and increased glomerular capillary pressure in the face of reduced ERPF implied that there must be an increase in efferent arteriolar tone leading to an increase in filtration fraction. Given the high prevalence of salt-sensitive hypertension in blacks, the documented rise in filtration fraction and intraglomerular pressure during high sodium intake suggests that these renal hemodynamic derangements might be partially responsible for the

greater propensity to hypertension-induced renal failure in this ethnic group (75). Thus, it remains possible that black patients have essential hypertension and that hypertension may lead to progressive renal injury in the absence of malignant hypertension or underlying primary renal disease. Even in high-risk populations, some 30% have been reported to have 0 ApoL1 risk alleles (130).

Experimental evidence in genetic models of hypertension supports this possibility. Renal function deteriorates faster in some strains of rats than others with genetic hypertension. In the SHR, hypertension is accompanied by an increase in renal afferent arteriolar resistance, thus protecting the glomeruli from the adverse effects of hypertension so that progressive renal insufficiency does not occur. In contrast, all salt-sensitive rat models of hypertension share the peculiarity of responding to a rise in BP with a decrease in afferent arteriolar resistance, which leads to an increase in glomerular capillary pressure with progressive renal injury (75).

Malignant Hypertension

Malignant hypertension is a distinct clinical and pathologic entity characterized by a marked elevation of BP (diastolic pressure often is >120–130 mm Hg) and evidence of widespread acute arteriolar injury (134). The clinical sine qua non of malignant hypertension is the funduscopic finding of *hypertensive neuroretinopathy*, which consists of striate (flame-shaped) hemorrhages, cotton wool (soft) exudates, and often papilledema. The development of hypertensive neuroretinopathy heralds the onset of a hypertensive vasculopathy that may cause necrotizing arteriolitis in the central nervous system, kidneys, and other vital organs. If the hypertension is untreated, then there is rapid and relentless progression to renal failure in less than 1 year, often with associated hypertensive encephalopathy, intracerebral hemorrhage, or congestive heart failure. Regardless of the degree of BP elevation, malignant hypertension cannot be diagnosed in the absence of hypertensive neuroretinopathy (134). There has been an unfortunate tendency in recent years to diagnose “malignant hypertension” in any patient with markedly elevated BP. However, given the prognostic and therapeutic implications of true malignant hypertension, it is extremely important to make a clear distinction between benign and malignant hypertension. This is not to say that benign hypertension cannot cause a hypertensive crisis. Benign hypertension that is accompanied by

acute end-organ dysfunction such as acute pulmonary edema, dissecting aortic aneurysm, or intracerebral bleeding clearly represents a hypertensive crisis requiring immediate reduction of the BP to avert disaster (134). On the other hand, marked elevation of BP frequently occurs in the absence of hypertensive neuroretinopathy or evidence of acute end-organ dysfunction. This entity, which is called *severe asymptomatic hypertension*, does not represent a true hypertensive crisis, and urgent treatment often is not required (134).

Headache and blurred vision are the most common presenting complaints in malignant hypertension. A striking "asymptomatic" presentation is not uncommon, especially in young black males who deny any prior symptoms when they present in the end stage of malignant hypertension with florid failure of the heart, brain, and kidney. In most patients, the diastolic pressure at presentation is >120 to 130 mm Hg. However, there is no absolute level of pressure above which malignant hypertension develops and there is considerable overlap of BP readings in patients with benign and malignant hypertension (134).

The patient with malignant hypertension (hypertensive neuroretinopathy) may or may not have clinically apparent end-organ involvement at the time of presentation. However, in the absence of adequate treatment, a variety of organ systems eventually will be damaged by the evolving hypertensive vasculopathy. Nervous system manifestations include hypertensive encephalopathy or intracerebral hemorrhage. Congestive heart failure with recurrent bouts of acute pulmonary edema is the most common cardiac complication. Gastrointestinal involvement may cause acute pancreatitis or an acute abdomen because of necrotizing mesenteric vasculitis. Patients with malignant hypertension may present with a spectrum of renal involvement ranging from minimal albuminuria with normal renal function to ESRD. In the untreated or inadequately treated patient, even if the renal function is initially normal, it is common to observe progressive deterioration to ESRD over several weeks to months (134).

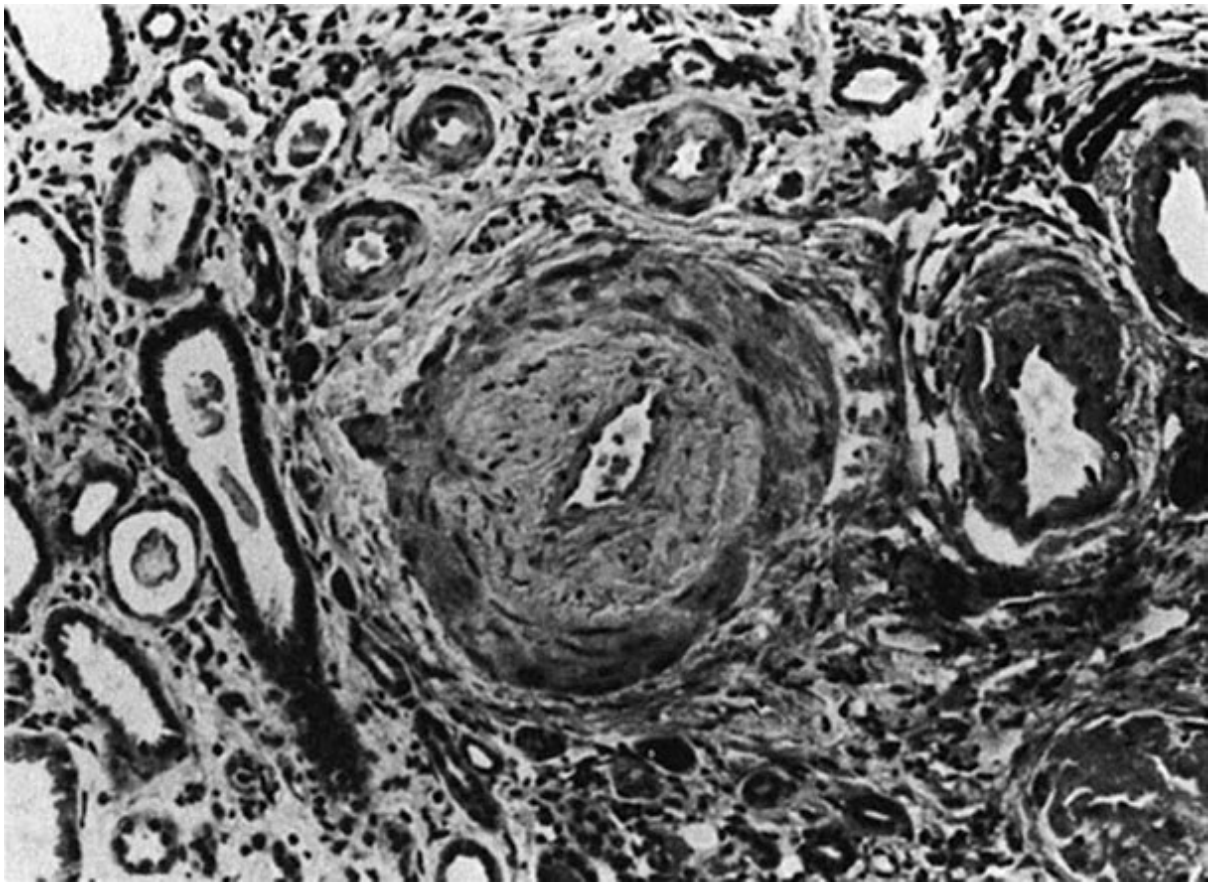

Figure 9-9 Musculomucoid intimal hyperplasia of an interlobular artery in malignant hypertension. The arterial wall is thickened by neointimal proliferation of myofibroblasts (modified smooth muscle cells), resulting in a significant reduction in the caliber of the arterial lumen. A small amount of myxoid material is seen between the smooth muscle cells (hematoxylin and eosin stain). (Reprinted from Pitcock JA, Johnson JG, Hatch FE, et al. Malignant hypertension in blacks: malignant arterial disease as observed by light and electron microscopy. *Hum Pathol*. 1976;7(3):333–346, with permission from Elsevier.)

PATHOLOGY OF MALIGNANT NEPHROSCLEROSIS

Even when terminal renal failure occurs in malignant hypertension, the kidneys may be normal in size. Small, pinpoint petechial hemorrhages on the cortical surface give rise to a peculiar, flea-bitten appearance. Fibrinoid necrosis of the afferent arterioles has traditionally been regarded as the hallmark of malignant nephrosclerosis. There is deposition in the media of a granular material that is pink with hematoxylin and eosin stain and a deep red color with trichrome stain (106). The characteristic finding in the interlobular arteries is severe luminal narrowing owing to intimal thickening (106). This lesion is known as proliferative endarteritis, endarteritis fibrosa, or the onionskin lesion. The arteriolar lumens are

severely narrowed because of thickening of the walls or superimposed fibrin thrombi. Focal and segmental fibrinoid necrosis was the predominant glomerular lesion in large autopsy series in the pretreatment era. However, accelerated glomerular obsolescence owing to ischemia is currently the most common finding at renal biopsy. In blacks with malignant hypertension, fibrinoid necrosis of the afferent arterioles is a rare finding. Instead, the afferent arterioles show a marked degree of hyalinization. The most prominent and characteristic finding is musculomucoid intimal hyperplasia of the interlobular arteries and larger arterioles (Fig. 9-9) (131). The intima of interlobular arteries is thickened by hyperplastic smooth muscle cells with variable degrees of fibrosis. The glomeruli show evidence of accelerated glomerular obsolescence with ischemic wrinkling of the glomerular basement membranes on electron microscopy (134).

PATHOPHYSIOLOGY OF MALIGNANT HYPERTENSION

The mechanism that initiates a transition from benign to malignant hypertension is unknown. Several pathophysiologic mechanisms have been postulated (134). According to the *pressure hypothesis*, the microvascular damage is a direct consequence of the mechanical stress placed on the vessel wall by hypertension. In contrast, the *vasculotoxic theory* holds that AII, vasopressin, and catecholamines not only raise BP but also induce direct vascular injury. Pressure-induced natriuresis with volume depletion and reflex activation of the RAS also may result in an unrelenting vicious cycle of hypertension and ischemic renal injury. The development of localized intravascular coagulation or altered metabolism of glucocorticoids, prostaglandins, or kininogens has been postulated to play a role in the acceleration of vascular injury.

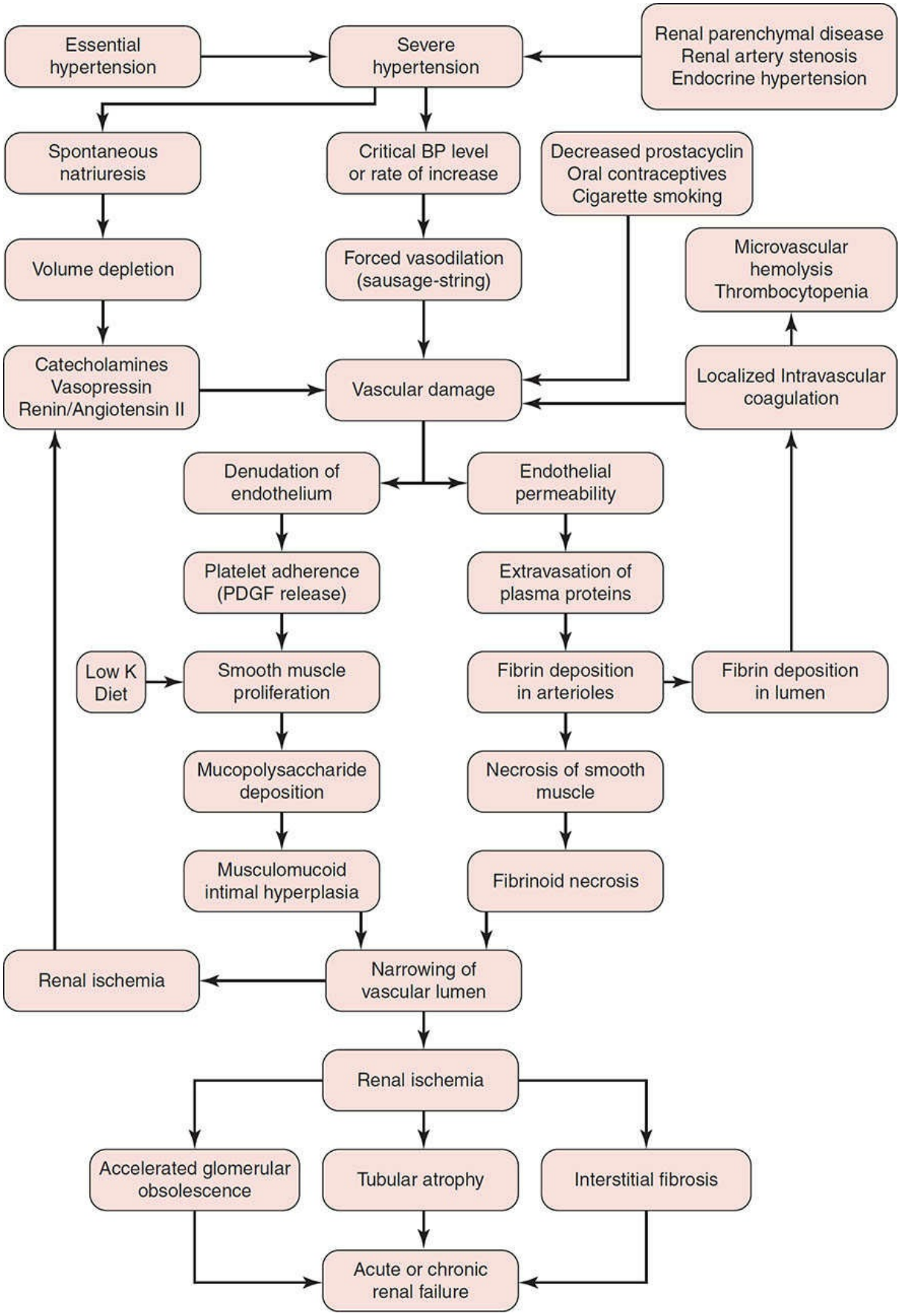

Figure 9-10 Pathophysiology of malignant hypertension. AII, angiotensin II; CHF,

congestive heart failure. (From Nolan CR, Linas SL. Malignant hypertension and other hypertensive crises. In: RW Schrier, ed. *Diseases of the Kidney and Urinary Tract*. 8th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2007;1370–1463.)

The vicious cycle of malignant hypertension is depicted in Figure 9-10. In the setting of severe essential hypertension or secondary hypertension, the pressure increases to a critical level or at a rate that overwhelms normal autoregulatory mechanisms and leads to focal areas of overstretched arterioles. The resulting endothelial damage allows extravasation of fibrinogen and other plasma proteins. The deposition of fibrin causes fibrinoid necrosis. Myointimal proliferation occurs, resulting in proliferative endarteritis. In the kidney, there is progressive glomerular injury owing to ischemia. Activation of the RAAS further increases the BP and leads to amplification of the cycle of hypertension and renal ischemia. The end result is renal failure. This widespread hypertensive vasculopathy also results in ischemic damage to other vascular beds. In the retina, ischemia of nerve fiber bundles leads to cotton wool spots and papilledema. Hypertensive neuroretinopathy occurs very early in the course of the disease and is the clinical hallmark of malignant hypertension.

RESPONSE TO TREATMENT IN MALIGNANT HYPERTENSION

In the absence of adequate BP control, malignant hypertension has a grave prognosis. In the preantihypertensive era, the 1-year mortality rate approached 90% and uremia was the most common cause of death. However, it is now clear that adequate treatment of essential hypertension prevents malignant hypertension. Furthermore, early and aggressive treatment of an established malignant phase prevents progressive renal damage. More severe renal dysfunction at presentation correlates with an increased risk of progression to ESRD. However, there are numerous reports of dramatic recovery of renal function in patients with malignant hypertension, even after months of renal failure requiring dialysis (134). This recovery of renal function has been attributed to strict BP control with the potent peripheral vasodilator minoxidil used in conjunction with a loop diuretic and a β-blocker. Presumably, the renal vasculopathy heals and the ischemic glomeruli recover when the inciting stimulus (severe hypertension) is removed.

Renovascular Hypertension

The landmark experimental models of hypertension developed by Goldblatt et al. demonstrated that persistent hypertension could be produced in dogs either by constricting both renal arteries or by removing one kidney and constricting the artery of the remaining kidney (135). Another variant of Goldblatt hypertension is produced by clipping the artery of one kidney and leaving the other kidney untouched. This two-kidney/one-clip (2K/1C) hypertension may be analogous to unilateral renal artery stenosis in humans. In this model, constriction of one artery leads to an immediate rise in BP because of increased renin production by the ischemic kidney. The activation of the RAS leads to AII-mediated vasoconstriction, impaired natriuresis in the ischemic as well as contralateral kidney and hypertension. Curiously, after a few days, even though BP continues to rise, the PRA returns to normal. In the early stages of 2K/1C hypertension, removal of the clipped kidney restores BP to normal. In contrast, later in the course, when the PRA is no longer elevated, the BP fails to normalize with angiotensin antagonists, removal of the clipped kidney, or unclipping. Of note, however, is the observation that removal of the contralateral "normal kidney" and unclipping normalizes BP. These findings imply that vascular changes that develop in the normal kidney when it is chronically exposed to elevated pressure may serve to perpetuate hypertension even after the original cause of the renovascular hypertension has been removed. Guyton's hypothesis implies that from the very beginning the contralateral kidney must have an abnormal renal function curve with a blunted natriuretic response to the elevated BP. Early in the course, this shift of the renal function curve may be mediated by functional changes induced by local intrarenal formation of AII. The direct and indirect effects of AII on renal sodium excretion have been discussed in detail in the preceding. The AII-dependent mechanisms that contribute to the development and maintenance of hypertension in the 2K/1C model probably act primarily by attenuating the ability of the animal to exhibit the expected hypertension-induced natriuresis by the contralateral (nonclipped) kidney. In later stages of renovascular hypertension, hypertension-induced structural damage may underlie the reduced natriuretic response to any given level of pressure. These secondary changes in the contralateral kidney may explain the well-known clinical observation that nephrectomy or revascularization for unilateral renal artery stenosis often fails to normalize BP. Preexisting essential hypertension also may explain the failure of revascularization to cure

hypertension in many cases.

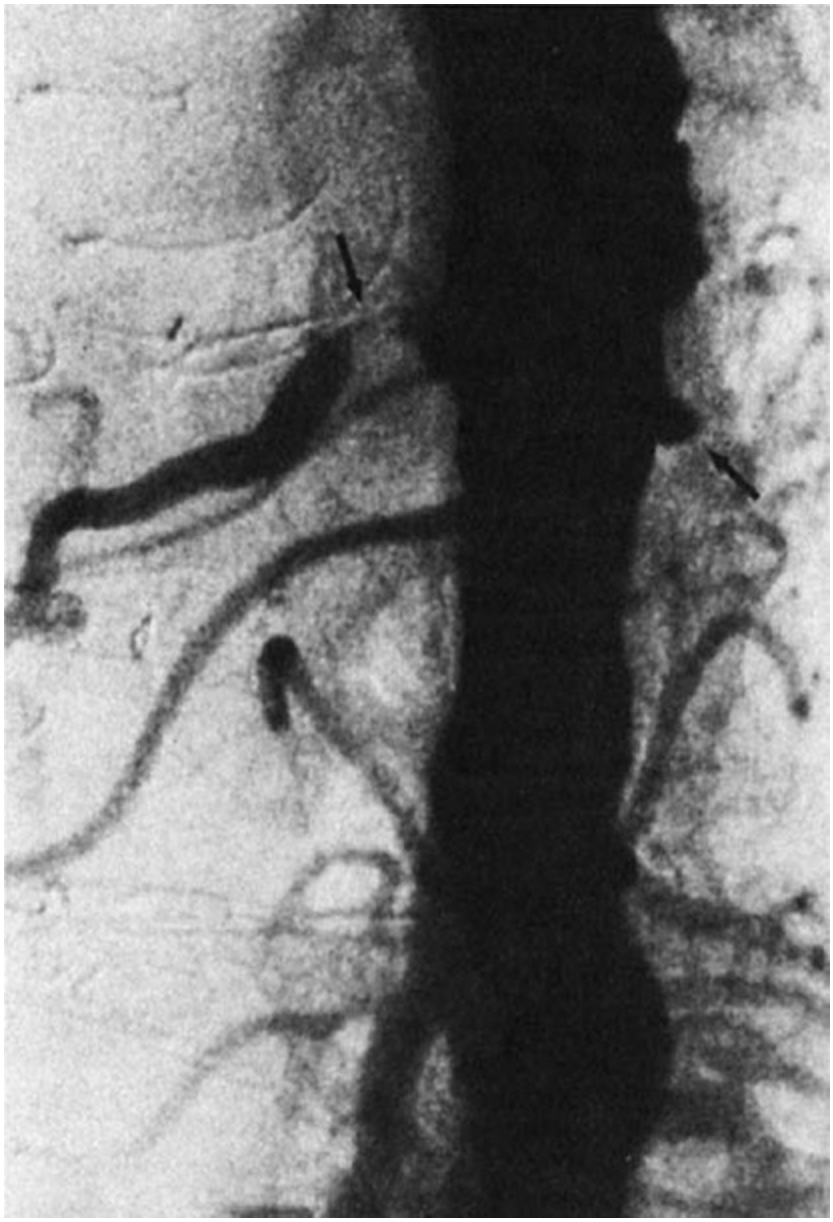

Figure 9-11 Aortogram demonstrating generalized atherosclerosis with bilateral atherosclerotic renal artery stenosis. The left renal artery is totally occluded at its origin. The right renal artery has a high-grade lesion near its origin (ostial lesion). (From Steven D. Brantley, MD, Department of Radiology, Wilford Hall Medical Center, Lackland Air Force Base, Texas, with permission.)

CAUSES OF RENAL ARTERY STENOSIS

The principal cause of renal artery stenosis is *atheromatous* narrowing of one or both main renal arteries (Fig. 9-11). Atheromatous renal artery stenosis occurs in older individuals, with a peak incidence in the sixth decade. Men are affected twice as often as women. It is most often found

in association with diffuse atherosclerotic disease of the aorta, coronary arteries, cerebral arteries, and peripheral vasculature. However, in 15% to 20% of cases, renal involvement occurs in the absence of atherosclerotic disease elsewhere (136). The obstructing atheromatous lesion is usually within the proximal 2 cm of the artery. Not uncommonly, a so-called ostial lesion is found with the lesion actually arising in the aorta at the origin of the renal artery.

A second type of arterial lesion, of obscure etiology, which affects the main renal artery is *fibromuscular dysplasia* (hyperplasia) (136). The lesion appears as a multifocal "string-of-beads" beginning in the mid-renal artery and often extending into peripheral branches (Fig. 9-12). This variant is typically seen in young to middle-aged women. The risk of progression to total arterial occlusion is small.

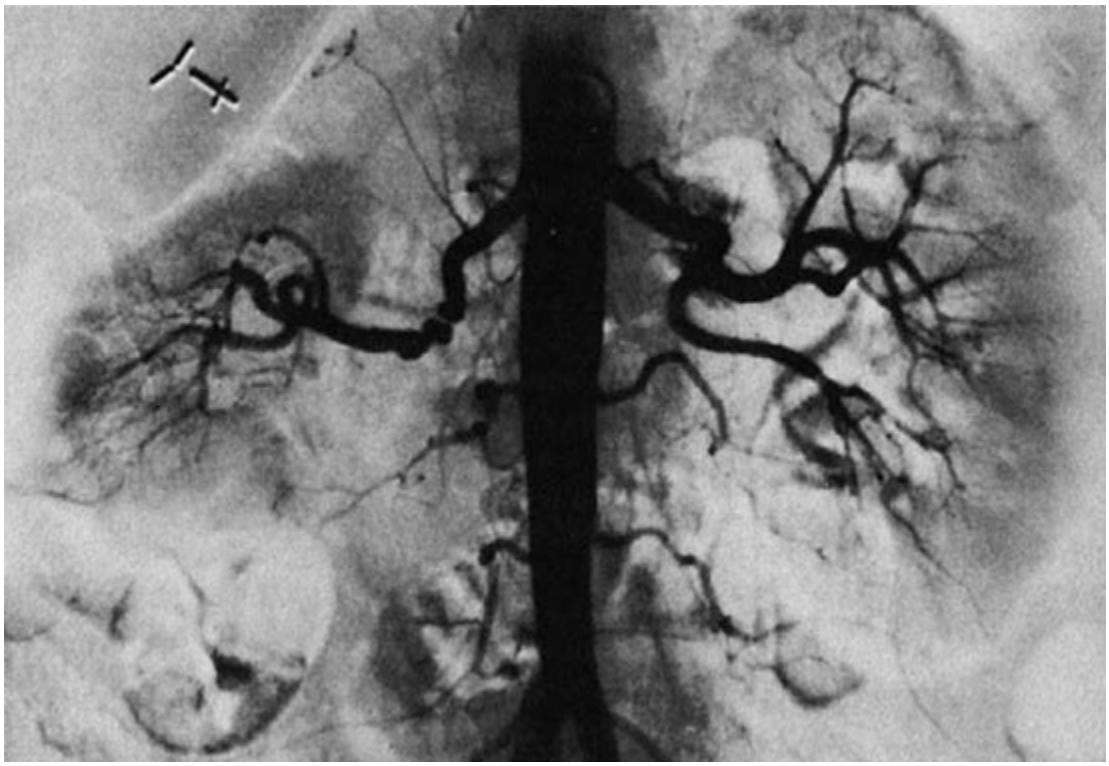

Figure 9-12 Renal angiogram demonstrating right renal artery stenosis owing to medial hyperplasia, the most common variant of fibromuscular dysplasia. The smaller right kidney has a mid-renal artery lesion with the characteristic "string of beads" appearance because of mural aneurysms, caused by thinning of the internal elastica, alternating with areas of narrowing caused by fibrovascular ridges. (From Steven D. Brantley, MD, Department of Radiology, Wilford Hall Medical Center, Lackland Air Force Base, Texas, with permission.)

SCREENING FOR RENOVASCULAR HYPERTENSION

Although renovascular hypertension owing to renal artery stenosis is one of the most common causes of potentially remediable secondary hypertension, available estimates suggest that <0.5% of the hypertensive population has renovascular hypertension (137). Thus, an aggressive approach to screening for this disorder often is not warranted. Several lines of evidence now suggest that an aggressive workup to exclude renovascular hypertension may not be cost-effective because the yield of curable hypertension is low, and the majority of patients can be managed successfully with medical therapy (137–139). The dilemma for the clinician lies in the fact that even the ideal screening test, with high sensitivity and specificity, has a low predictive value when applied indiscriminately to the general hypertensive population where the prevalence of renovascular hypertension is low. In this regard, aggressive screening leads to the generation of many more false-positive results (essential hypertension) than true-positive results (occult renovascular hypertension). This statistical phenomenon undoubtedly accounts for the numerous highly touted screening tests that have come and gone over the years. The rapid sequence intravenous pyelogram (hypertensive IVP) is no longer routinely used as a screening tool because it is not only insensitive but also has a false-positive rate of up to 12% among patients with essential hypertension (137). Isotope renography (renal scan) has proved even less accurate than the hypertensive IVP because of an unacceptable frequency of false-positive results in patients with essential hypertension (137). Casual measurements of PRA are of little value (137). The highly touted “captopril test,” which measures the increase in venous PRA in response to captopril, proved to have low specificity (137).

Thus, it is apparent that sound clinical judgment is essential in the selection of patients in whom an aggressive evaluation for renovascular hypertension is indicated. Certain clinical clues suggest the possibility of underlying renovascular hypertension (108). The onset of hypertension before the age of 30 years should suggest secondary hypertension. A truly abrupt onset of hypertension at any age suggests renal vascular disease. However, more often than not, this finding represents newly diagnosed essential hypertension rather than recently developed secondary hypertension. A definite worsening of previously well-controlled hypertension should suggest renovascular hypertension. Clues on physical examination include the finding on funduscopy of striate hemorrhages, cotton wool spots, or papilledema (malignant hypertension) (134), or a continuous systolic and diastolic epigastric bruit.

Even in the presence of diffuse atherosclerotic disease, aggressive

evaluation for renovascular hypertension is only indicated if the BP is truly resistant (>150/100 mm Hg) to a rationale triple-drug regimen that includes a diuretic (137). Unexplained deterioration of renal function despite adequate BP control suggests the possibility of ischemic nephropathy and should prompt a search for bilateral renal artery stenosis with ischemic nephropathy (140). Deterioration of renal function on the addition of an ACE inhibitor or angiotensin receptor blocker (ARB) suggests the possibility of bilateral renal artery stenosis or stenosis of a solitary kidney. This phenomenon probably reflects maintenance of GFR in the ischemic kidneys by AII-mediated vasoconstriction of the efferent arteriole to increase filtration fraction.

In the patient in whom the probability of renovascular hypertension is high, conventional screening tests are of little value because the predictive value of a negative test is low (138). Computerized tomographic angiography and gadolinium-enhanced three-dimensional magnetic resonance angiography appear to be the best noninvasive tests for detection of anatomic renal artery stenosis (141). Nonetheless, intraarterial contrast angiography remains the gold standard for definitive diagnosis of renal artery stenosis (137). Unfortunately, selective renal angiography carries definite risks including contrast media-associated nephrotoxicity and renal atheroembolic disease (142). Further complicating this issue is the fact that anatomic renal artery stenosis does not always imply functional renovascular hypertension. Incidental renal artery stenosis can clearly occur in essential hypertension. Selective renal vein renin determinations have been used to predict the functional significance of anatomic lesions. A renal vein renin ratio greater than 2:1 (involved to uninvolved) was highly predictive of a beneficial response to intervention. One study suggested that a change in the Tc 99m-labeled diethylene triamine pentaacetic acid (DTPA) or mercapto acetyl tri glycine (MAG_3) renogram after treatment with captopril may help to define the functional significance of a renal artery lesion before intervention with surgery or angioplasty (143).

MEDICAL THERAPY VERSUS ANGIOPLASTY FOR TREATMENT OF RENAL ARTERY STENOSIS

Renal artery stenosis is not an uncommon finding in patients with evidence of atherosclerotic disease elsewhere in the body. In fact, incidental renal angiography in patients undergoing coronary angiography demonstrates renal artery stenosis in 6% to 18% of patients (144,145). Likewise, renal

artery stenosis is found in 16% to 40% of patients undergoing aortography for aortic aneurysms or peripheral vascular disease (146,147). The clinical utility of renal revascularization via angioplasty and stenting or bypass surgery has been controversial. In this regard, three randomized clinical trials of renal artery angioplasty compared with medical therapy alone had shown no convincing benefit of angioplasty with regard to BP control (148–150). A subsequent review was conducted on the basis of analysis of the MEDLINE database from inception to September 2005 for studies involving adults with atherosclerotic renal artery stenosis that reported morality, renal function, BP, cardiovascular events, or adverse events (151). The authors concluded that available evidence does not clearly support renal revascularization over intensive medical therapy for atherosclerotic renal artery stenosis. However, the issue was best addressed by several randomized controlled clinical trials. In the Angioplasty and Stent for Renal Artery Lesions (ASTRAL), 806 patients with atherosclerotic renovascular disease were randomized to undergo revascularization in addition to medical therapy versus medical therapy alone (152). The primary outcome was renal function with secondary outcomes being BP, the time to renal and major cardiovascular events, and mortality. During the 5-year follow-up period, there was no difference in either primary or secondary outcomes. There was, however, a higher risk of adverse events with revascularization including two deaths and three amputations of toes or limbs, suggesting that revascularization was associated with significant risk but no clinical benefit (152). Similar findings were reported in a smaller study—Stent Placement in patients with Atherosclerotic Renal Artery Stenosis (STAR) (153). Here 140 individuals with kidney disease and significant RAS were randomized to receive stenting and medical therapy versus medical therapy alone. The primary outcome, progression of kidney disease, was similar between both groups at the end of the study duration (16% of participants in each group achieved 20% reduction in creatinine clearance). Serious complications were noted here including two procedure-related deaths. More recently, in the Cardiovascular Outcomes in Renal Atherosclerosis Lesions (CORAL), a multicenter trial, 947 patients were randomized to medical therapy alone versus treatment with angioplasty and nondrug-eluting stents plus intensive medical therapy. Patient were followed up for a median of 43 months. Outcome measures included the incidence of myocardial infarction (MI), heart failure, strokes, and renal failure. The CORAL trial results indicated no significant difference between both study groups in rates of any of the individual components of the primary outcome or in the

composite end point. The study furthermore showed no difference in all-cause mortality (154). Collectively and overwhelmingly, the existing evidence opposes revascularization interventions for atherosclerotic renovascular hypertension.

Hypertension due to Primary Aldosteronism

Primary aldosteronism resulting from an adrenocortical adenoma (aldosteronoma), originally described by Conn, is another of the few potentially curable causes of secondary hypertension (155). *Aldosteronoma* are usually small (<2 cm diameter) benign adrenal nodules, which account for 70% to 80% of cases of primary aldosteronism. *Idiopathic aldosteronism*, which is associated with bilateral micronodular or macronodular adrenal hyperplasia, accounts for 20% to 30% of cases of primary aldosteronism. The prevalence of primary aldosteronism in unselected patients with hypertension is low, on the order of 1% to 2% (149). The clinical features of primary aldosteronism are the result of the effects of aldosterone on renal sodium handling. Aldosterone binds and activates the intracellular MR in principal cells in the DCT resulting in an increase in the number of open ENaCs in the luminal membrane with enhanced sodium reabsorption. The resulting impairment in the natriuretic capacity of the kidney causes salt-sensitive hypertension. Despite the fact that renal sodium handling in the distal tubule is abnormal, patients with primary hyperaldosteronism lack edema or evidence of increased ECF volume. This paradox results from the fact that the kidney escapes from the salt-retaining effects of a mineralocorticoid via a pressure natriuresis and diuresis that returns ECF volume to normal. Chronic hypertension in this setting is maintained by an increase in systemic vascular resistance perhaps via a guytonian mechanism. The electronegative potential of the distal tubule lumen favors secretion of potassium and hydrogen ion leading to hypokalemia and metabolic alkalosis in some, but not all, patients. PRA is suppressed in almost all patients with primary aldosteronism reflecting the state of relative volume expansion. However, PRA is also suppressed in patients with low-renin essential hypertension, so measurement of PRA alone is not a reliable method of screening.

Given the relatively low prevalence of primary hyperaldosteronism in the general hypertensive population, routine screening for this disorder is not recommended. Screening should generally be reserved for hypertensive patients who have spontaneous hypokalemia (not due to

diuretics or other secondary causes of hyperaldosteronism) or profound diuretic-induced hypokalemia or patients with severe or resistant hypertension (155). Recent data suggest that determining the ratio of PAC (ng/dL) to PRA (ng/dL/h) in a subject with untreated hypertension is the most acceptable screening method for distinguishing patients with essential hypertension from those with primary aldosteronism (155,156). The timing of the test (morning), the posture of the patient before blood sampling (upright), and the units of measure should be standardized. If the cutoff value of this ratio is kept high enough (e.g., >30 or 50) the test will be sensitive enough to identify most cases of primary aldosteronism while maintaining reasonable specificity. The mean ratio for normal subjects and patients with essential hypertension is 4:10. An elevated PAC/PRA ratio alone does not establish the diagnosis of primary aldosteronism. Further testing is mandatory to establish a definitive biochemical diagnosis based on documentation of nonsuppressible aldosterone secretion during sodium loading and renin hyporesponsiveness to sodium depletion. Aldosterone suppression testing can be performed either with orally administered sodium chloride and measurement of 24-hour urine aldosterone secretion or with intravenous saline loading and measurement of PAC. Before testing, hypokalemia should be corrected because it suppresses aldosterone secretion. If the withdrawal of antihypertensive medications is not feasible, BP should be treated with CCBs, β-blockers, or α-blockers that do not affect diagnostic accuracy. ACE inhibitors, ARBs, and spironolactone should be stopped because they interfere with the tests. Oral sodium loading (2–3 g sodium chloride/day) is performed by placing the patient on a high-salt diet with supplemental sodium chloride tablets if needed for 3 days. Vigorous replacement of potassium chloride also should be prescribed because sodium loading increases kaliuresis. On the third day of the diet, serum electrolytes are measured and a 24-hour urine is collected for measurement of aldosterone, sodium, and potassium. Adequate sodium loading is present if the 24-hour urine sodium exceeds 200 mEq. Urine aldosterone excretion >14 μg/day is consistent with hyperaldosteronism. Alternatively, aldosterone suppression can be performed by intravenous administration of 2 L of normal saline over 4 hours while the patient is recumbent. The PAC will fall to a level <6 ng/dL in patients with essential hypertension, whereas values >10 ng/dL are consistent with primary aldosteronism. Inability to stimulate renin should then be confirmed by placing the patient on a low-sodium diet (40 mg/day) or treatment with a diuretic (furosemide, up to 120 mg/day in divided doses). The PRA should remain below 1 ng/dL/h in patients with primary

hyperaldosteronism.

Once the biochemical diagnosis of primary aldosteronism has been confirmed, the approach to therapy depends on whether the disease is caused by aldosteronoma or idiopathic aldosteronism. Computerized tomography (CT) can detect most aldosteronoma >1 cm in size. Unfortunately, CT results can be misleading. A nodule <1 cm in size may be undetected, leading to an erroneous diagnosis of idiopathic aldosteronism. On the other hand, in patients with idiopathic aldosteronism, the adrenal glands are usually either normal in size or bilaterally enlarged. However, the fortuitous presence of an incidental nonfunctional adrenal nodule may lead to an erroneous diagnosis of aldosteronoma. Selective adrenal vein sampling during corticotropin stimulation, with simultaneous measurement of PAC and cortisol is invasive, but represents a much more reliable method to differentiate aldosteronoma from idiopathic aldosteronism (155,156). Measurement of cortisol as well as aldosterone in the adrenal veins and inferior vena cava is critical for evaluating the accuracy and success of adrenal venous sampling. A unilateral excess of aldosterone secretion suggests the presence of aldosteronoma.

Patients with aldosteronoma are best treated with removal of the affected adrenal gland, which dramatically reduces or cures hypertension in the majority patients. The BP response to spironolactone is predictive of the response to surgery in patients with aldosteronoma but not those with idiopathic aldosteronism (155). Spironolactone therapy for 3 to 4 weeks preoperatively is useful to allow for repletion of total body potassium and minimize postoperative hypoaldosteronism. Laparoscopic surgery is now widely employed to resect aldosteronoma and other adrenal tumors. On the other hand, idiopathic aldosteronism is best managed medically, because unilateral or bilateral adrenalectomy fails to normalize BP and patients then need lifelong glucocorticoid and mineralocorticoid replacement in addition to antihypertensive therapy. High-dose spironolactone, an aldosterone antagonist, is the mainstay of therapy that can be used in conjunction with other antihypertensive agents such as CCBs.

GRA (also known as dexamethasone-suppressible hyperaldosteronism or familial hyperaldosteronism type I), is a rare genetic form of hyperaldosteronism caused by an autosomal dominant mutant chimeric gene that drives constitutive aldosterone synthesis under the control of ACTH and independent of volume status and AII (52). The administration of exogenous glucocorticoids that suppress ACTH secretion results in suppression of aldosterone secretion, reversal of the mineralocorticoid

state, and resolution of hypertension. Unlike other etiologies of primary aldosteronism, which are usually diagnosed in the third to fifth decades of life, GRA is evident from birth onward. Hypertension associated with GRA is often difficult to control with conventional antihypertensive agents. The diagnosis of GRA should be considered in a patient with early-onset hypertension, especially in childhood or a history of early-onset hypertension in first-degree relatives. Another clue is a prominent family history of hemorrhagic stroke at a young age. There is an increased prevalence of cerebral hemorrhage, which occurs at a mean age of 32 years, and is associated with a 60% mortality rate. Some patients with GRA fit the classical description of a mineralocorticoid-excess state, including hypertension, hyporeninemia, and spontaneous hypokalemia. However, analysis of large GRA pedigree has shown that affected patients often are normokalemic except during treatment with potassium-wasting diuretics. Thus, evaluation for spontaneous hypokalemia lacks sensitivity as a screening test for GRA. Patients with GRA have a PAC–PRA ratio >30. The diagnosis of GRA is supported by dexamethasone suppression testing (DST). A fall in PAC to 4 ng/dL after low-dose DST (0.5 mg dexamethasone orally every 6 hours for 2–4 days) is sensitive and specific for the diagnosis of GRA. The adrenal cortex in GRA produces large quantities of 18-oxygenated cortisol compounds, 18-oxocortisol (18-oxo-F), and 18-hydroxycortisol (18-OH-F), so-called hybrid steroids because they possess enzymatic features of both zona glomerulosa and zona fasciculata steroids. Significantly elevated levels of these hybrid compounds in a timed 24-hour urine specimen therefore provide a highly sensitive and specific test to diagnose GRA. Genetic testing for the chimeric gene is 100% sensitive and specific to diagnose GRA (52). GRA can be treated with long-term glucocorticoid suppression; however, glucocorticoid therapy has significant long-term complications, especially in children. For this reason, monotherapy with spironolactone (a competitive antagonist of the MR) or amiloride (blocks the aldosterone-regulated ENaC) represents the preferred treatment for GRA. Cerebral hemorrhage in GRA results from intracranial aneurysm; therefore, routine screening of GRA patients with magnetic resonance angiography is recommended, beginning at puberty and every 5 years thereafter (52).

Hypertension Caused by Glucocorticoid Excess in Cushing Syndrome

Glucocorticoid excess may overwhelm the capacity of 11β-HSD to convert cortisol to cortisone in distal tubular cells so that cortisol is available to bind to the MR. The resulting enhancement of renal sodium reabsorption leads to salt-sensitive hypertension. Cushing syndrome most commonly results from overproduction of ACTH either owing to pituitary adenoma or ectopic secretion of the hormone by a nonpituitary tumor. Hypokalemic metabolic alkalosis also may result from activation of the MR. The purpose of the low-dose dexamethasone suppression test is to differentiate patients with Cushing syndrome from those with normal function of the hypothalamic–pituitary axis. Failure of the morning serum cortisol to fall to <140 nmol/L following a single midnight dose of dexamethasone (1 mg) suggests the presence of Cushing syndrome. The high-dose dexamethasone suppression test then is used to differentiate patients with Cushing disease (pituitary hypersecretion of ACTH) from those with ectopic secretion of ACTH.

Hypertension in Chronic Kidney Disease

Virtually all forms of primary renal parenchymal disease can lead to secondary hypertension, especially if renal insufficiency is present (113,118). Glomerulonephritis and vasculitis are more likely to cause hypertension than chronic interstitial nephritis. Hypertension is present in >75% of cases of acute poststreptococcal glomerulonephritis. In a series of patients with biopsy-proven glomerulonephritis, the overall prevalence of hypertension was 60%. Hypertension was found more commonly with IgA nephropathy, membranoproliferative glomerulonephritis, and focal segmental glomerulosclerosis, whereas it was less frequent with membranous nephropathy or minimal change disease. In the setting of lupus nephritis, the frequency of hypertension approaches 50%. In idiopathic rapidly progressive (crescentic) glomerulonephritis, hypertension is uncommon unless overt fluid overload is present. Hypertension is extremely common in diabetic glomerulosclerosis. In fact, most patients with type 2 diabetes have long-standing hypertension as part of the metabolic syndrome–insulin resistance syndrome. Autosomal dominant polycystic kidney disease (ADPKD) is associated with a >50% incidence of hypertension even before the onset of renal insufficiency. Recent studies have found that the incidence of hypertension in ADPKD may be related to the degree of renal cyst enlargement (157).

A variety of disorders of the renal vasculature other than stenosis of the

main renal arteries also may produce hypertension. Systemic vasculitis owing to classic polyarteritis nodosa is frequently accompanied by hypertension that may enter a malignant phase. In patients with progressive systemic sclerosis, hypertension plays a central role in the precipitous loss of renal function that occurs with scleroderma renal crisis (158). Thrombotic microangiopathy owing to hemolytic uremic syndrome or thrombotic thrombocytopenic purpura also can cause severe hypertension. Renal cholesterol embolization syndrome following an angiographic procedure in patients with severe aortic atherosclerosis can cause sudden onset of severe hypertension that may enter a malignant phase (159).

The prevalence of hypertension increases with progressive chronic renal insufficiency, regardless of cause, so that at end stage, virtually all patients are hypertensive. Among patients with ESRD, roughly 70% have hypertension owing to volume overload, and hemodialysis alone normalizes BP. Approximately 30% of patients have dialysis-resistant hypertension, which may be due to hyperactivity of the RAAS or sympathetic nervous system, and thus require long-term antihypertensive therapy (160). Hypertension is also extremely common in the renal transplant recipient and may result from a variety of factors including acute or chronic rejection, stenosis of the transplant renal artery, calcineurin inhibitors (cyclosporine and tacrolimus), high-dose glucocorticoids, or increased renin production by diseased native kidneys (161).

ROLE OF HYPERTENSION IN THE PATHOGENESIS OF DIABETIC NEPHROPATHY

In diabetic patients, hypertension is a major risk factor for large-vessel atherosclerotic disease affecting the coronary, cerebral, and peripheral vascular beds. The incidence of large-vessel disease is dramatically increased in both type 1 and type 2 diabetics and is a major cause of morbidity and premature death. Diabetic patients have a twofold increased risk of coronary artery disease, a twofold to sixfold increased risk of atheroembolic stroke, and a significantly increased risk of peripheral vascular disease (162). Hypertension also hastens the progression of diabetic microangiopathic complications such as nephropathy and retinopathy (162). In non–insulin-dependent (type 2) diabetes mellitus (NIDDM), hypertension is twice as common as in the nondiabetic population. This increased prevalence of hypertension may relate to

underlying insulin resistance, one of the sequelae of which is impairment in the intrinsic natriuretic capacity of the kidney. Moreover, control of hypertension, especially in conjunction with the use of ACE inhibitors and ARBs, has been shown in clinical trials to slow the progression of diabetic nephropathy (163–165).

The evolution of hypertension in insulin-dependent diabetes mellitus (IDDM, type 1) has been well characterized (166). In the early years of type 1 diabetes, hypertension is no more common than in healthy controls. Incipient nephropathy (microalbuminuria) is accompanied by a small but consistent increase in BP compared with controls. With the development of overt diabetic nephropathy (clinically apparent proteinuria), hypertension is the rule, and the severity of the hypertension correlates inversely with the level of renal function. Once the serum creatinine begins to increase, the prevalence of hypertension is >90%. In contrast, in the older patient with NIDDM (type 2) diabetes, the natural history of hypertension is less predictable. Essential hypertension is likely to coexist in a substantial proportion of patients even before the development of nephropathy. As discussed in detail, the presence of hyperinsulinemia, which is present long before overt hyperglycemia develops, may lead to impaired natriuresis with salt-sensitive essential hypertension.

Numerous experimental and clinical observations suggest an important role for hypertension in the progression of diabetic nephropathy. Hypertension is thought to accelerate diabetic renal injury by exacerbating the underlying abnormalities in renal hemodynamics and further elevating glomerular capillary flow and pressure. Poor glycemic control may lead to afferent arteriole vasodilation, thereby allowing enhanced transmission of the elevated systemic BP to the glomeruli. Diabetic patients destined to develop nephropathy have a higher prevalence of hypertension and a higher mean BP than diabetic patients not destined to develop nephropathy. Moreover, there is a threefold increase in the risk of nephropathy among type 1 diabetics with a parental history of hypertension, suggesting that an inherited predisposition to essential hypertension may increase the risk of nephropathy (167). In a multivariate analysis of patients with overt diabetic nephropathy, more rapid loss of GFR correlated most strongly with higher DBP (168). In contrast, blood sugar control, assessed by hemoglobin A_1C, did not correlate with change in GFR, at least in these patients with overt nephropathy.

In a study of the effect of two-kidney/one-clip Goldblatt hypertension in the streptozotocin-induced diabetic model in rats, severe diabetic nephropathy was observed in the unclipped kidney exposed to the high

systemic pressure, whereas in the clipped kidney, protected from the high systemic pressure, nephropathy did not develop (169). This experimental demonstration of the central role of hypertension in the pathogenesis of diabetic nephropathy is further supported by reports of autopsy findings in patients with diabetes and unilateral renal artery stenosis. Glomerular basement thickening and nodular Kimmelstiel–Wilson lesions were found only in the kidney with the patent renal artery and thereby exposed to the high systemic arterial pressure (170).

In rat models, diabetic nephropathy is produced by injection of streptozotocin with maintenance of moderate hyperglycemia with low-dose insulin. Initially there is a substantial increase in whole-kidney GFR akin to the hyperfiltration observed in young juvenile diabetic patients. There is also an increase in single nephron GFR owing to intrarenal vasodilation of both afferent and efferent arterioles, which results in an increase in glomerular capillary pressure and flow. However, after several weeks, progressive proteinuria, hypertension, and glomerulosclerosis develop (120). As in the remnant kidney model, it has been proposed that this increase in glomerular hydraulic pressure is maladaptive and eventually leads to progressive renal injury and nephron loss. In this model, normalization of glomerular capillary pressure with chronic ACE inhibitor therapy prevents the development of proteinuria and glomerulosclerosis, supporting the important pathophysiologic role of glomerular capillary hypertension. In these models of diabetic nephropathy, ACE inhibitors appear to be superior to conventional triple antihypertensive therapy with a thiazide diuretic, reserpine, and hydralazine. It has been proposed that this is caused by a selective decrease in efferent arteriolar tone with ACE inhibitors, which leads to a direct reduction in glomerular capillary pressure independent of a reduction in systemic pressure (120).

TREATMENT OF HYPERTENSION IN DIABETIC PATIENTS

There is now convincing evidence from clinical trials that ACE inhibitors are beneficial in the treatment of diabetic nephropathy through a mechanism that is independent of their effect on systemic BP. The Collaborative Study Group investigated the effect of ACE inhibitors in type 1 diabetics, 18 to 49 years old, with overt diabetic nephropathy (proteinuria ≥500 mg/day) (163). All subjects had serum creatinine levels ≤2.5 mg/dL. For the 75% of patients who were hypertensive on entering the study, treatment was instituted as required with antihypertensive

medications other than ACE inhibitors or CCBs. Subjects (200 each group) were then randomized to receive either captopril (25 mg) or placebo tablets given three times a day for a median of 3 years. The BP goal during the study was <140/90 mm Hg. Over the course of the study, MAPs were generally slightly lower (<4 mm Hg) in the ACE inhibitor-treated group. The primary end point of the study was a doubling of the baseline serum creatinine that was reached in 25 subjects in the captopril group and 43 in the placebo group (P = 0.007). Captopril treatment reduced the relative risk of doubling serum creatinine by 48%. Captopril treatment was also associated with a 50% reduction in the relative risk of ESRD disease or death. Overall, in the captopril group, the mean rate of increase in serum creatinine was 0.2 ± 0.8 mg/dL/y versus 0.5 ± 0.8 mg/dL/y in the placebo group. Since the inclusion of MAP as a time-dependent covariate in the statistical analysis did not alter the risk reduction estimates, it was concluded that there was a specific beneficial effect of ACE inhibitors independent of systemic BP reduction. Recent studies also suggest that ACE inhibitors are useful in normotensive type I diabetic patients with incipient diabetic nephropathy (microalbuminuria), in that they slow the increase in microalbuminuria and reduce the probability of progression to clinical proteinuria (166).

The recent Irbesartan in Diabetic Nephropathy Trial (IDNT) has confirmed a renoprotective effect of ARBs in patients with diabetic nephropathy owing to type 2 diabetes (159). In this trial, 1,715 hypertensive patients with nephropathy caused by type 2 diabetes were randomly assigned to treatment with irbesartan (300 mg/day), the CCB amlodipine (10 mg/day), or placebo. BP was controlled to a target of 135/85 mm Hg with the addition of antihypertensive agents of classes other than ARB/ACE inhibitor or CCBs if necessary. Treatment with irbesartan was associated with a 20% (compared with placebo) or 23% (compared with amlodipine) reduction in the risk of development of the composite end point of doubling of baseline serum creatinine, development of ESRD, or death from any cause. The risk of doubling of serum creatinine was 33% lower in the irbesartan group than in the placebo group and 37% lower than in the amlodipine group. The relative risk of ESRD was 23% lower with irbesartan than with either amlodipine or placebo. These differences were not explained by differences in the achieved level of BP control between the groups. It is possible that the dihydropyridine CCBs (e.g., amlodipine or nifedipine) by causing preferential dilatation of the afferent arteriole may allow more aortic pressure to be transmitted to the glomeruli. Thus, the reduction in systemic

BP produced by these CCBs may not be accompanied by a corresponding reduction in intraglomerular pressure. This may well be the pathophysiologic mechanism underlying the variable effect of dihydropyridine CCBs on proteinuria and progression of diabetic renal disease.

In the RENAAL trial (Reduction of End points in NIDDM with the Angiotensin II Antagonist Losartan), 1,513 hypertensive type 2 diabetic patients with proteinuria were randomized to losartan 50 to 100 mg/day and followed up for >3 years (165). The investigators reported a significant reduction in the incidence of ESRD as well as the end point of doubling of serum creatinine.

Given the diverse effects of the RAS on the kidney, ACE inhibitors and ARBs may have protective effects in diabetic nephropathy in addition to glomerular hemodynamic effects. Postulated protective mechanisms include improvement in glomerular permselectivity with decreased proteinuria, decreased mesangial matrix expansion, inhibition of glomerular hypertrophy, amelioration of insulin resistance, improvement in serum lipid profiles, changes in AII-mediated renal sodium handling, inhibition of renal procollagen formation, and inhibition of atherogenesis (120). Moreover, there is increasing recognition of the fact that even in primary glomerular diseases such as diabetic nephropathy, the decrement in GFR correlates best with the extent of interstitial disease (tubular atrophy and interstitial fibrosis). The link between glomerular disease and interstitial disease may be explained by the fact that proteinuria leads to increased protein catabolism by tubular epithelial cells, which in turn increases the expression of TGF-β. Expression of fibrogenic cytokines therefore might provide a link between proteinuria and the development of interstitial fibrosis. In this regard, therapeutic maneuvers, such as BP reduction or use of ACE inhibitors, which reduce proteinuria might slow progression of renal disease by modulating TGF-β-mediated interstitial damage.

Clinically, it has been shown that effective antihypertensive therapy with a diuretic and a β-blocker can reduce proteinuria and slow the progression of renal disease in patients with established diabetic nephropathy (171). At least in retrospective studies, the correlation between DBP and rate of progression of diabetic nephropathy is valid even at pressures <90 mm Hg (168). This observation suggests that the usual therapeutic target for DBP may be too high and that patients may benefit from reductions of BP into the low–normal range. In this regard, one study had demonstrated that aggressive BP control in normotensive type 2

diabetic patients is beneficial (172). In this study, normotensive type 2 diabetic patients were randomized to intensive (10 mm Hg below the baseline DBP) versus moderate DBP control (80–89 mm Hg). Patients in the moderate group were given placebo, whereas patients in the intensive group were randomized to treatment with either ACE inhibitor (enalapril) or CCB (nisoldipine). Over the 5-year follow-up period, compared to patients in the moderate group, intensive BP control (average BP 128/75 mm Hg) with either drug slowed the progression to incipient (microalbuminuria) and overt (macroalbuminuria) diabetic nephropathy. Intensive BP control also decreased the progression of diabetic nephropathy and diminished the incidence of stroke. More recently, the Action to Control Cardiovascular Risk in Diabetes Study (ACCORD) examined the question of intensive BP control in patients with type 2 DM. In a multicenter study, 4,733 individuals with type 2 DM and normal creatinine and normoalbuminuria were randomized to intensive versus standard BP control and followed up for 4.7 years (173). The primary outcome was a composite of nonfatal MI, nonfatal stroke, and death from cardiovascular disease. SBP in the standard control arm was 133.5 mm Hg and in the intensive control arm 119.3 mm Hg after 1 year. There was no difference in the composite primary outcome between both groups although similar to the preceding trial, intensive BP control did reduce the risk of stroke. In addition, no significant difference was noted between both groups for kidney disease progression.

MECHANISMS OF HYPERTENSION IN CHRONIC KIDNEY DISEASE

In acute nephritic syndrome due to poststreptococcal glomerulonephritis, hypertension is clearly caused by sodium and water retention with increased ECF volume, plasma volume, and cardiac output with either normal or increased systemic vascular resistance (113,118). In contrast, the cause of hypertension in the setting of chronic renal insufficiency owing to primary renal disease is more controversial. It has been postulated that hypertension is caused by volume expansion with inappropriately increased renin release. However, in both humans and animal models with hypertension owing to primary renal disease, the ECF volume, plasma volume, and cardiac output are usually normal, whereas hypertension is maintained by an increased systemic vascular resistance. Nonetheless, sodium intake clearly plays an important role in the genesis of hypertension because BP is much more sodium-sensitive in patients with

chronic renal insufficiency than in normal subjects (174).

The genesis of hypertension in the setting of primary renal disease can be readily conceptualized in the framework of either the Na/K ATPase inhibitor or Guyton hypotheses. Declining nephron mass is associated with a diminished capacity to excrete a sodium load. A compensatory increase in Na/K ATPase inhibitor may cause an increase in systemic vascular resistance and thus hypertension (Fig. 9-2). On the other hand, Guyton suggests that in the face of this type of primary natriuretic defect, the renal fluid–volume feedback mechanism restores external sodium balance, but does so at the expense of systemic hypertension that is maintained by an increase in peripheral vascular resistance secondary to the autoregulatory response (Fig. 9-5) (23–25,92).

Hypertension caused by intrinsic renal disease also may be related to the activation of renal pressor mechanisms such as the RAAS in ADPKD (157) or diminished production of vasodilator substances (bradykinins, prostaglandins, and NO) (118). The demonstration that ninefold increases in circulating inhibitors of NO synthesis may occur in uremic patients implies that deficiencies of vasodilatory substances may indeed be an important contributor to the elevated systemic vascular resistance in hypertensive patients with renal failure (175).

ROLE OF HYPERTENSION IN THE PROGRESSION OF CHRONIC KIDNEY DISEASE

In the setting of primary renal parenchymal disease, hypertension clearly has its origin in the kidney. There is now substantial clinical and experimental evidence to support the concept that this secondary hypertension in turn aggravates the underlying disorder and is a major factor in the progression of chronic renal insufficiency (176). Coexistence of hypertension and primary renal disease creates a vicious circle, hastening the progression of renal failure. Systemic hypertension traditionally had been assumed to accelerate primary renal disease by inducing structural damage in the renal microvasculature (hyaline arteriosclerosis) with resultant glomerular hypoperfusion and ischemia (Fig. 9-13). However, it is now widely accepted that the converse may be true, namely, that systemic hypertension induces progressive injury in the already diseased kidneys via hydraulic stress on the glomeruli caused by increased transmission of the elevated systemic pressure to the glomerular capillaries (hyperperfusion theory) (Fig. 9-13) (121,177). The differences in the pathophysiologic mechanism of renal injury between essential

hypertension (hypoperfusion/ischemia) and secondary hypertension caused by primary renal disease (hyperperfusion/glomerular hypertension) may be explained by differences in afferent arteriolar resistance in these disorders. Afferent arteriolar resistance determines the fraction of the systemic pressure transmitted to the glomerular capillaries. In benign essential hypertension, structural changes in the afferent arterioles increase resistance and presumably serve to prevent transmission of the elevated systemic pressure to the glomeruli. In contrast, glomerular hemodynamic changes in the setting of primary renal disease may be entirely different. The most extensively studied model of hypertension in the setting of a reduced number of normally functioning nephrons is the *remnant kidney* model produced by surgical ablation of renal mass in the rat. Reduction in renal mass below a critical level is associated with the development of proteinuria, systemic hypertension, and progressive renal failure owing to glomerulosclerosis in the remnant kidney (178). The reduction in functioning nephrons leads to a compensatory increase in single nephron GFR in the remaining nephrons. Vasodilatation of both the afferent and efferent arterioles leads to a decrease in renal vascular resistance with a resulting increase in glomerular capillary plasma flow. Glomerular capillary pressure increases because the decrease in efferent resistance is less pronounced than the decrease in afferent resistance. Together the increases in glomerular capillary pressure and perfusion account for the observed compensatory single nephron hyperfiltration. Similar glomerular hemodynamic changes are observed in the deoxycorticosterone acetate (DOCA)-salt hypertension model and the salt-sensitive model of nephrotoxic serum nephritis (179). Moreover, deterioration of renal function accelerates dramatically when secondary hypertension owing to DOCA-salt administration or renal artery clipping is superimposed on immune complex or nephrotoxic serum nephritis (179). Brenner et al. have suggested that these compensatory increases in glomerular capillary flow and pressure, although sufficient to maintain whole-kidney GFR in the short term, are maladaptive in the long term, and that the resulting hydraulic stress is somehow responsible for the eventual development of glomerulosclerosis in the remaining nephrons (121,178). Micropuncture studies have confirmed that in the diseased kidneys autoregulation is lost and afferent arterioles are dilated so that the elevated systemic pressure is transmitted to the glomeruli, resulting in glomerular capillary hypertension, which is thought to induce progressive renal injury (Fig. 9-13).

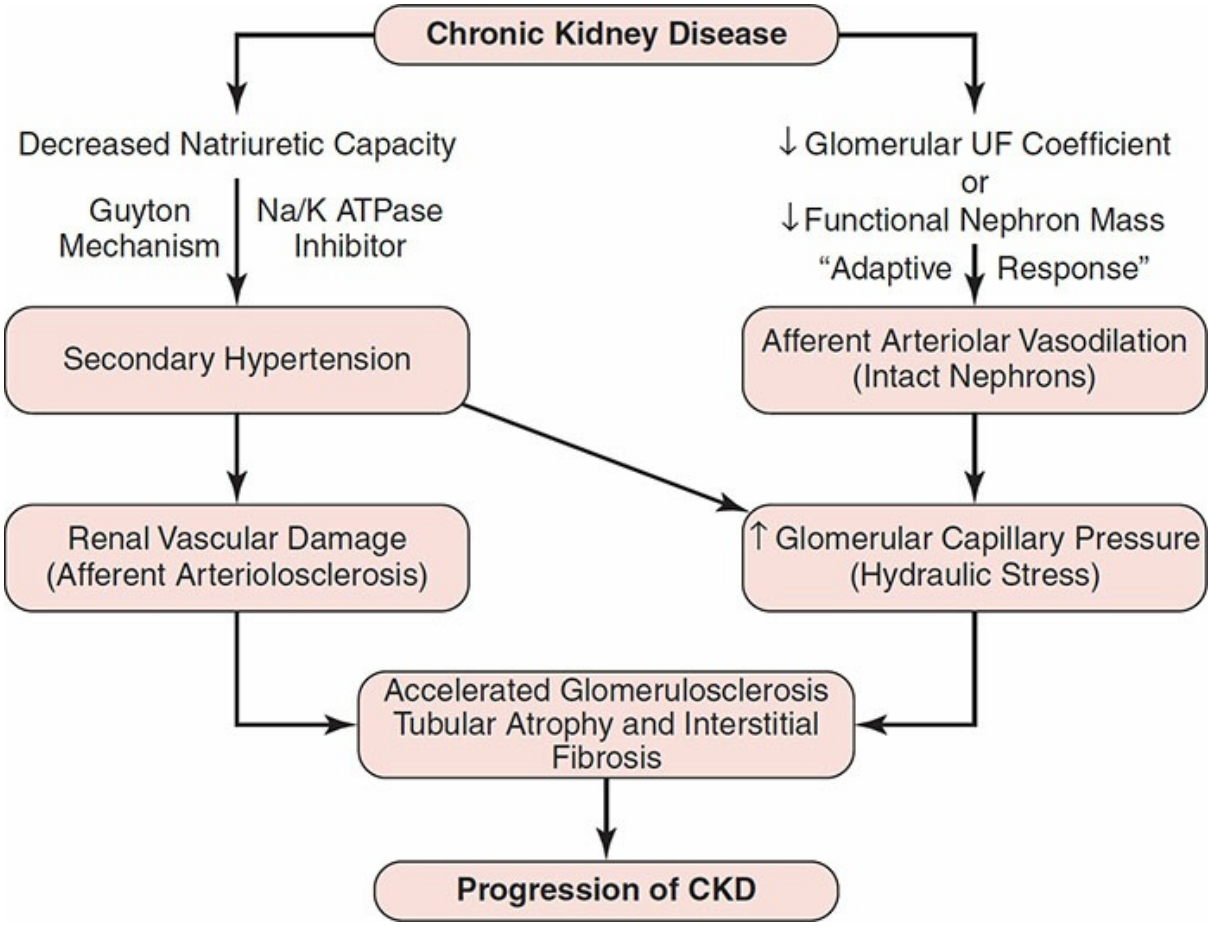

Figure 9-13 Role of hypertension in the progression of chronic kidney disease. Secondary hypertension may contribute to the inexorable progression of primary renal disease by either of two mechanisms. Hypertensive damage to the renal microvasculature may lead to hyaline arteriolosclerosis of the afferent arteriole with further renal injury induced by an ischemic mechanism. Alternatively, following nephron loss caused by the primary disease, compensatory responses in the remaining nephrons cause afferent arteriolar vasodilation. Although this hemodynamic response helps to maintain whole-kidney glomerular filtration rate (GFR), in the long term it may be maladaptive. The decrease in afferent arteriolar resistance allows for transmission of the elevated systemic arterial pressure to the glomeruli. The hemodynamic stress caused by elevated glomerular capillary flow and pressure causes accelerated glomerulosclerosis and progression of chronic renal insufficiency. UF, ultrafiltration.

In the preceding models, treatment of hypertension is associated with a slowing of the progression of renal injury (180). However, in the renal ablation model, although both ACE inhibitor (enalapril) and triple therapy (reserpine, hydralazine, and hydrochlorothiazide) reduced BP equally, only ACE inhibitor treatment ameliorated proteinuria and glomerular scarring (181). The superiority of ACE inhibitor therapy in these models has been attributed to the fact that it leads to a reduction in intrarenal AII-mediated efferent arteriolar tone, thereby directly reducing glomerular capillary

pressure in addition to lowering systemic BP. We previously discussed the clinical use of ACE inhibitors and ARBs to slow the progression of renal disease in diabetic nephropathy.

Nonetheless, systemic hypertension and glomerular hypertension do not always coexist. A maladaptive increase in glomerular capillary flow and pressure may be a phenomenon that occurs principally in the setting of reduced functioning renal mass owing either to ablation or intrinsic renal disease. Systemic hypertension occurs without the development of glomerular capillary hypertension in the SHR (182). The glomeruli are protected from the high systemic pressure by afferent arteriolar vasoconstriction, which may explain the absence of progressive renal dysfunction in this model. The critical role that afferent arteriolar tone plays in the protection against hypertensive glomerular injury is illustrated by the fact that the reduction in nephron mass by unilateral nephrectomy in the SHR results in a reduction in afferent arteriolar resistance in the remaining kidney. This allows for transmission of the systemic pressure to the glomeruli and, in this model, progressive glomerular injury with glomerulosclerosis does occur (183). The observation may be pertinent to understanding the low risk of progressive loss of renal function in benign essential hypertension in humans. In benign hypertension there is also a relative intrarenal vasoconstriction. It is tempting to speculate that, at least in patients with salt-resistant essential hypertension, there may be an increase in afferent arteriolar resistance that protects the glomeruli from the deleterious effects of systemic hypertension and thus accounts for the infrequent occurrence of progressive renal insufficiency among white patients with essential hypertension.

TREATMENT OF HYPERTENSION TO SLOW DISEASE PROGRESSION IN NONDIABETIC CHRONIC KIDNEY DISEASE

Evidence is accumulating that strict control of BP is also beneficial in slowing the rate of progression of nondiabetic CKD. Furthermore, animal and human studies have shown that progression of CKD may be exacerbated by secondary hemodynamic factors such as intraglomerular hypertension. Therefore, a vital component of the treatment of hypertension in patients with CKD, especially those with proteinuria, is the administration of an ACE inhibitor as part of a multidrug regimen not only to optimally control BP but also with the goal of slowing the progressive loss of renal function. In the Benazepril trial, patients already in reasonable

BP control were randomized to treatment with benazepril or placebo. Patients on benazepril had a greater reduction in BP and a 25% reduction in protein excretion (184). The risk of progression to a primary end point (doubling of serum creatinine or progression to dialysis) was reduced by 53% in the benazepril-treated patients. The benefits of ACE inhibitor therapy were seen mainly in patients with chronic glomerular diseases or diabetic nephropathy, whereas there was no benefit in patients with polycystic kidney disease or other CKD excreting <1 g of protein per day (two settings in which hemodynamically mediated factors may not be as important in disease progression). In the Ramipril Efficacy in Nephropathy (REIN) trial, patients with nondiabetic renal disease were randomized to ramipril or placebo plus other antihypertensive therapy as need to achieve DBP below 90 mm Hg (185). The trial was terminated prematurely among patients excreting >3 g protein per day because of a significant benefit with ACE inhibitor treatment with regard to ameliorating the rate of decline of renal function. The AASK was designed to address the efficacy of different classes of antihypertensive agents (*β*-blocker, ACE inhibitor, dihydropyridine CCB) in slowing the progressive loss of renal function in blacks with benign hypertensive nephrosclerosis (186). Use of diuretic therapy was allowed as necessary in each treatment groups to achieve target BP goals. The National Institutes of Health (NIH) called a premature halt to the CCB arm of the study when interim analysis by the independent data safety monitoring board revealed that patients with hypertensive nephrosclerosis and proteinuria exceeding 300 mg/day benefited more from treatment with an ACE inhibitor (ramipril) than CCB (amlodipine) (187). Results of the AASK trial suggested that an ACE inhibitor (ramipril) was more effective in slowing the progression of benign hypertensive nephrosclerosis in blacks than either amlodipine or metoprolol (186). Although the final results of the AASK trial showed no difference among the drug treatment groups in the rate of decline of GFR, the ramipril group had a 22% reduction in risk of the composite end point (reduction in GFR by >50% from baseline, ESRD, or death). In the REIN-2 trial, a dihydropyridine channel blocker (CCB) failed to provide renoprotection in patients with nondiabetic renal disease, despite further reduction in BP from that obtained with fixed doses of ACE inhibitors (188). The nondihydropyridine CCBs (diltiazem and verapamil) have antiproteinuric effects, whereas the dihydropyridines (amlodipine and nifedipine) have been shown to increase proteinuria in some studies. This paradox may be explained by the varied effect of the different classes of CCBs on renal autoregulation. In this regard, dihydropyridines cause

preferential afferent arteriolar dilatation that allows more of the systemic pressure to be transmitted to the glomerulus, thereby increasing glomerular pressure and limiting their antiproteinuric effect.

The Kidney Disease Outcomes Quality Initiative (K/DOQI) work group on hypertension and antihypertensive agents in CKD recommends that either an ACE inhibitor or an ARB should be used as first-line antihypertensive therapy in proteinuric patients with nondiabetic CKD (189). Although the available evidence is strongest for ACE inhibitors, ARBs may be substituted in patients who develop cough during treatment with ACE inhibitors. Although the issue is not as well studied in nondiabetic renal disease, ARBs appear to have similar antiproteinuric activity compared to ACE inhibitors and also significantly slow disease progression in patients with type 2 diabetes and nephropathy. A multidrug antihypertensive regimen is usually required to achieve the goal of reduction in BP below 130/80 mm Hg. Recent long-term follow-up of AASK indicates the importance of this strategy in African Americans with hypertensive nephrosclerosis. Among patients followed for 8.8 to 12.2 years, individuals with proteinuric CKD with a protein:creatinine (P:C) ratio >0.22 CKD progression was delayed with intensive BP control (the intensive arm achieved BP 130/78 during the trial phase) (190). If this BP goal is not achieved after initial therapy with an ACE inhibitor or an ARB, a diuretic should be added to the regimen. Addition of a diuretic is logical therapy given the central role of impaired natriuresis in the pathogenesis of hypertension in the setting of CKD. Thiazide diuretics may be effective in the early stages of CKD, whereas loop diuretics may be necessary in patients with more advanced kidney disease or diuretic resistance in the setting of nephrotic syndrome. If the BP goal is not achieved with combination ACE inhibitor and diuretic therapy, additional drugs may be added to the regimen including a β-blocker, or a nondihydropyridine CCB (diltiazem or verapamil). Hydralazine or minoxidil (in combination with appropriate doses of β-blocker to control heart rate and diuretic to prevent fluid retention) may be added to the regimen in patients with resistant hypertension.

Treatment of Hypertension in Dialysis Patients

In the initial era of maintenance hemodialysis therapy in the early 1960s, Scribner et al. convincingly demonstrated that combining long hemodialysis sessions with assiduous attention to dietary sodium

restriction resulted in normalization of BP in >90% of hemodialysis patients (191,192). Unfortunately, in recent decades, progressive shortening of dialysis sessions, administration of higher sodium dialysate to avoid intradialytic hypotension, and lack of attention to the utility of strict dietary sodium restriction have all collectively resulted in a progressive loss of BP control in dialysis patients (Fig. 9-14) (191). As a result, the vast majority of dialysis patients now require a multidrug regimen to adequately control BP. The key to normalization of BP in dialysis patients without the need for antihypertensive drugs is meticulous attention to the achievement of true dry weight. Charra has defined dialysis dry weight as "the post-dialysis weight at which the patients remains normotensive until the next dialysis session despite modest interdialytic fluid retention and without the need for antihypertensive medications" (191,193). In the absence of residual renal function, there are three basic modalities for control of ECF volume in dialysis patients: dietary sodium restriction, removal of sodium into the dialysate by diffusion (with lower sodium dialysate), and convective losses of sodium by ultrafiltration. Incredibly, most dialysis providers tend to emphasize "fluid restriction" to the patient as the primary means to prevent excessive interdialytic fluid gains, while the value of dietary sodium restriction has been forgotten and is all too often neglected in patient education. In reality, it is excessive dietary sodium intake that is the primary driving force for the typically large interdialytic fluid gains. In this regard, if fluid intake per se were actually the central issue, patients with impressive interdialytic weight gains would present with profound hyponatremia. In fact, even patients with 10-kg weight gains tend to have serum sodium concentrations in the low–normal range. This observation regarding isotonic extracellular volume expansion implies that these patients have actually ingested both salt and water in isotonic proportions. In this regard, a dialysis patient presenting with a 10-kg weight gain and serum sodium of 135 mEq/L has indeed imbibed 10 L of fluid but has also ingested 31 g of sodium. Since the ingested sodium is the factor driving excessive thirst, patient education regarding 'fluid restriction" alone is virtually never effective. In contrast, the interdialytic weight gain can be significantly reduced by moderate sodium restriction (2 g sodium or 5–6 g NaCl per day) without emphasis on the need for fluid restriction per se (191). A reasonable dialysate sodium concentration (135–138 mmol/L) will also provide for some diffusive loss of sodium during dialysis. Convective removal of sodium by ultrafiltration is the third arm of the triad for achieving true dry weight on dialysis. In this regard, long slow dialysis such as that provided by home

daily nocturnal dialysis is particularly effective at achieving true dry weight and normalization of BP without the need for antihypertensive medications (194).

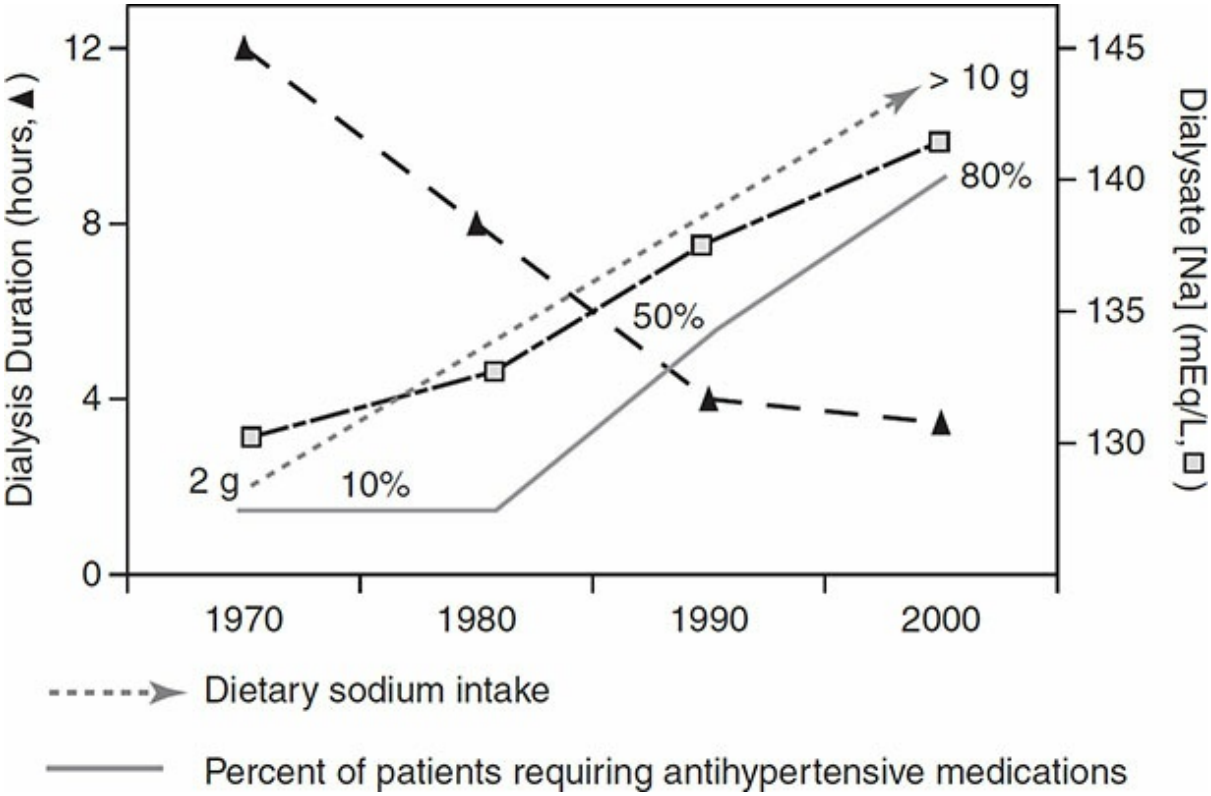

Figure 9-14 Chronological trends in dialysis session time, dialysate sodium concentration, dietary sodium intake, and prevalence of hypertension in dialysis patients. In the initial era of maintenance dialysis treatment in the 1960s, prescription of long dialysis session times, moderately low dialysate sodium concentrations, and meticulous attention to dietary sodium restriction resulted in normalization of blood pressure (BP) in 90% of dialysis patients without the need for antihypertensive medications. Over the ensuing decades, changes in the dialysis prescription, focusing on adequacy of dialysis as defined by urea kinetics, have led to progressively shorter dialysis sessions and increasing use of higher dialysate sodium concentrations and sodium modeling to avoid interdialytic hypotension. Nephrologists have also "forgotten" the importance of restriction of sodium intake as the prime modality for prevention of large intradialytic weight gains, while inappropriately shifting the focus of patient education to "fluid restriction." The net result is that the majority (>80%) of dialysis patients fail to achieve true dry weight and remain hypertensive despite "adequate" dialysis. With current dialysis practice, >80% of patients require antihypertensive medication (often a multidrug regimen) to adequately control BP (*triangles* = dialysis session time; *squares* = dialysate sodium concentration [mmol/L]; *dashed line* = dietary sodium intake [g/day]; *triple line* = percentage of dialysis patients requiring antihypertensive medications). (From Charra B. Fluid balance, dry weight, and blood pressure in dialysis. *Hemodialysis Int.* 2007;11:21–31, with permission from John Wiley and Sons.)

THIAZIDE DIURETICS IN THE TREATMENT OF ESSENTIAL HYPERTENSION

Because impaired renal handling of sodium plays a central role in the pathogenesis of both essential and secondary forms of hypertension, the use of diuretics is a well-established approach in the treatment of hypertension. Diuretics have been employed for the treatment of hypertension since the discovery of chlorothiazide in 1957. Over the last 40 years, the efficacy of thiazides and related diuretics in preventing complications of essential hypertension has been conclusively demonstrated in long-term controlled clinical trials (195–198). Mild to moderate hypertension often responds to treatment with low-dose thiazide diuretics alone (4). In the setting of severe hypertension, or with concomitant renal insufficiency, diuretics are often an essential element of a stepped-care, multidrug regimen (4). Moreover, the failure to include a diuretic in the antihypertensive regimen is a common cause of "resistant" hypertension that fails to respond to treatment with ACE inhibitors, CCBs, α-adrenergic blockers, β-blockers, or vasodilators used singly or in combination (199).

Thiazide diuretics bind to and inhibit the thiazide-sensitive NCC in the DCT (200). It is interesting to note that the physiologic mechanism that underlies pressure natriuresis also involves downregulation of the NCC (105). The acute effect of diuretic administration is a decrease in ECF and plasma volume with a reduction in cardiac output. However, over a period of days to weeks of chronic thiazide diuretic administration, the negative sodium balance is attenuated such that plasma volume and cardiac output are normalized. Nevertheless, the antihypertensive effect persists, implying a concomitant decrease in systemic vascular resistance. This secondary *decrease in systemic vascular resistance* appears to be the long-term mechanism of the antihypertensive action of thiazides and related diuretics (201–203). The precise mechanism of this reduction in systemic vascular resistance is unknown. However, in the framework of either the Na/K ATPase inhibitor or Guyton hypothesis, a reduction in systemic vascular resistance and BP as an indirect result of the natriuretic action of the diuretics is easy to conceptualize. As discussed, an underlying defect in natriuretic capacity must be present in hypertension, regardless of etiology. Diuretic treatment, by at least partially ameliorating this defect in

natriuresis, could result in a decrease in the circulating level of the Na/K ATPase inhibitor, which has been proposed as the cause of the increase in vascular reactivity and systemic vascular resistance in hypertension (Fig. 9-2). In the context of Guyton's hypothesis, restoration of natriuretic capacity toward normal with diuretics means that the renal fluid–volume feedback mechanism no longer necessitates the presence of systemic hypertension (increased systemic vascular resistance) to maintain sodium balance (Fig. 9-5).

SAFETY OF THIAZIDE DIURETICS FOR TREATMENT OF HYPERTENSION

During the past two decades, there has been a gradual shift away from diuretics as first-line antihypertensive agents toward the preferential use of newer agents such as converting enzyme inhibitors, CCBs, and selective α-adrenergic blockers. A major cause for concern has been related to the potentially deleterious metabolic effects of diuretics (hypokalemia, hypomagnesemia, hyperuricemia, glucose intolerance, and increased cholesterol). Fortunately, it now appears that the use of low-dose thiazide diuretics (12.5–25 mg/day of hydrochlorothiazide, or equivalent) has antihypertensive efficacy similar to high-dose diuretic therapy, while markedly reducing the incidence of metabolic abnormalities. In addition, concern has been expressed that thiazide diuretics may aggravate cardiac arrhythmias and increase the complications of coronary heart disease, including MI and sudden death. However, the HDFP study revealed a significant reduction at 8-year follow-up in all-cause mortality in its intensively (high-dose diuretic) treated stepped-care group relative to the referred care control group (190). There was a 16% risk reduction for fatal ischemic heart disease. This difference was noted primarily in the fatal MI classification, in which there was a 23% risk reduction. In the European Working Party on High Blood Pressure in the Elderly trial, a double-blind placebo-controlled trial of low-dose hydrochlorothiazide plus triamterene in patients over the age of 60 years, total cardiovascular mortality was reduced by 38%, and deaths from MI were reduced by 60% (197). In the Systolic Hypertension in the Elderly Program (SHEP) trial, a double-blind placebo-controlled trial of low-dose chlorthalidone in patients over the age of 60 years with isolated systolic hypertension, the relative risks of stroke, left ventricular failure, nonfatal MI or fatal coronary heart disease, and requirement for coronary artery bypass grafting were all significantly reduced in the active treatment group (198).

CHOICE OF DRUGS FOR THE TREATMENT OF HYPERTENSION

It is currently advisable in clinical practice to follow the latest recommendations of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC 8 Hypertension Guideline Algorithm) (5), which advocates beginning treatment in uncomplicated essential hypertension in nonblack individuals with one of the following options: ACE inhibitor, ARB, thiazide diuretic, or CCB. For black individuals with essential hypertension, the guideline recommends initial treatment with a thiazide diuretic alone or in combination with a CCB. Treatment should be individualized. Thiazide diuretics provide a safe, effective, and inexpensive form of treatment in the vast majority of hypertensive patients. However, in some patient groups, drugs other than diuretics may be considered as first-line therapy (5). For instance, given the evidence that ACE inhibitors and ARBs have a beneficial effect in slowing the progression of diabetic nephropathy, they should be considered the initial antihypertensive drug of choice for the treatment of diabetic patients with hypertension. Nonetheless, ACE inhibitor or ARB therapy alone may not adequately control BP and the addition of other classes of antihypertensives may be required. In diabetic patients with resistant hypertension or overt diabetic nephropathy with nephrotic syndrome, the use of thiazide or loop diuretics may be imperative. Given their proven benefits with regard to slowing progression of nondiabetic CKD, ACE inhibitors also should be included as part of the antihypertensive regimen in patients with chronic renal disease and proteinuria exceeding 1 g/day. ACE inhibitors also should be considered as initial therapy for hypertensive patients with congestive heart failure caused by systolic dysfunction. In hypertensive patients with prior MI, β-blockers should be considered first-line therapy because they reduce the risk of reinfarction and sudden death (5). In the setting of hypertension with coexistent renal insufficiency, addition of diuretics is often required to treat refractory hypertension. Substitution of the more potent, loop diuretics may be required in patients failing to respond to thiazide diuretics (i.e., GFR <25 mL/minute). In patients with severe benign hypertension or malignant hypertension, a triple-drug regimen, employing a diuretic and β-blocker in conjunction with the use of a potent peripheral vasodilator such as hydralazine or minoxidil, may be required to achieve adequate BP control (5).

The flexibility in choice of first-line agent for the treatment of

uncomplicated essential hypertension stems from available data. Outcome studies support the conclusion that the achieved level of BP, rather than the class of drug used, is the principal determinant of overall benefit. In this regard, the majority of trials indicate that at the same level of BP control, most antihypertensive drugs provide equivalent cardioprotection. For example, the Captopril Prevention Project (CAPPP) randomized trial (204), the Swedish Trial in Old Patients with Hypertension-2 (STOP-Hypertension-2) study (205), the Nordic Diltiazem (NORDIL) study (203), the Intervention as a Goal in Hypertension Treatment (INSIGHT) study (206), and the Antihypertensive and Lipid-Lowering to Prevent Heart Attach Trial (ALLHAT) (207) each found no significant difference in overall mortality between older classes of drugs (thiazide-like diuretics or *β*-blockers) and newer antihypertensive drugs (ACE inhibitors or CCBs). The major exception to this generalization is selective *α*-blocker therapy, which has been found to be associated with a higher risk of heart failure in high-risk hypertensive patients (208). For this reason, selective *α*-blockers (prazosin and doxazosin) are not recommended as first-line antihypertensive therapy. The principal finding from ALLHAT is that chlorthalidone (thiazide diuretic), amlodipine (CCB), and lisinopril (ACE inhibitor) provided similar protection from coronary heart disease death and nonfatal MI (207). In fact, thiazide diuretic was actually superior to CCB and/or ACE inhibitor in preventing some adverse cardiovascular events. For instance, development of heart failure was significantly less common with chlorthalidone than with either lisinopril or amlodipine. Furthermore, there was a significantly increased risk of stroke and combined cardiovascular disease among black patients given lisinopril compared to those treated with chlorthalidone. An unexpected finding was that lisinopril failed to provide an advantage for any outcome measure when compared with chlorthalidone. Indeed, lisinopril-treated patients had higher combined cardiovascular disease outcomes, stroke, and heart failure than chlorthalidone-treated patients. Remarkably, chlorthalidone was even superior to lisinopril among diabetic patients with regard to the prevention of new onset heart failure and the combined cardiovascular disease outcome. Thus, results from the ALLHAT strongly suggest that initial therapy with a thiazide diuretic is the appropriate choice for most hypertensive patients.

With regard to combination therapy, the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) trial was designed to test the hypothesis that treatment with an ACE inhibitor combined with amlodipine would

result in better cardiovascular outcomes than treatment with the same ACE inhibitor combined with a thiazide diuretic (209). In this randomized double-blind trial, 11,056 patients with hypertension who were at high risk of cardiovascular events were assigned to receive treatment with either benazepril plus amlodipine or benazepril plus hydrochlorothiazide. The primary end point was the composite of death from cardiovascular causes, nonfatal MI, nonfatal stroke, and hospitalization for angina, resuscitation after sudden cardiac arrest, and coronary revascularization. Mean BP was similar in both groups. At 36 months of mean follow-up, there were 552 primary outcome events in the benazepril–amlodipine group (9.6%) and 679 in the benazepril–hydrochlorothiazide group (11.8%), representing an absolute risk reduction with benazepril–amlodipine therapy of 2.2% and a relative risk reduction of 19.6% (hazard ratio 0.08, 95% confidence interval, 0.72–0.90, $P < 0.002$). For the secondary end point of death from cardiovascular causes, nonfatal MI, and nonfatal stroke, the hazard ratio was 0.79 (95% CI, 0.67–0.92, $P = 0.002$). The authors conclude that the benazepril–amlodipine combination was superior to the benazepril–hydrochlorothiazide combination in reducing cardiovascular events in patients who were at high risk of such events.

In older adults (>60 years of age), JNC 8 recommends treatment of hypertension when the BP is 150/90 or higher (5). These recommendations preceded and are in contrast to the findings of the SBP Intervention Trial (SPRINT) (210). The trial was conducted in 9,361 individuals at least 50 years of age with SBP 130 mm Hg or more (and without DM) who were randomized to standard (<140 mm Hg) versus intensive (<120 mm Hg) BP targets. The mean SBP was 121.4 and 136.2 mm Hg in the intensive and standard arms, respectively. The intervention was stopped after 3.26 years due to a significantly lower rate of cardiovascular events (fatal and nonfatal) in the intensive BP group. The hazard ratio for the composite primary outcome was 0.75 (95% CI, 0.64–0.90) in the intensive control arm compared to the standard arm. The results of this study may inform future guidelines and should be weighed by clinicians in the care of patients with essential hypertension.

Conclusions

It is apparent that the kidney is both the villain and the victim in hypertension. The kidney has a central role in the pathogenesis of both essential hypertension and secondary hypertension caused by primary

renal parenchymal disease, renal artery stenosis, and mineralocorticoid excess. Analysis of rare mendelian causes of hypertension has provided convincing evidence that abnormal renal sodium handling plays a central role in the pathogenesis of hypertension. Ongoing genetic linkage analysis studies may provide additional insights into the pathogenesis of essential hypertension and hopefully lead to the development of novel treatments to ameliorate the impairment in natriuretic capacity that characterizes both primary and secondary hypertension of all causes (16). At present, there is little hard evidence to support the widely held notion that benign essential hypertension is a common cause of ESRD although it is an important risk factor for cardiovascular disease and needs to be treated aggressively. Recent data indicate genetic disposition among black individuals for kidney disease associated with hypertension and suggest that hypertensive nephrosclerosis is a multifactorial disease. Although essential, hypertension by itself is not a likely cause of ESRD. Without question, hypertension does play a critical role in accelerating the progression of CKD. Rigorous control of BP with a regimen including an ACE inhibitor or ARB has been shown to slow the progression of both diabetic and nondiabetic renal disease. Large outcome trials have produced conflicting results regarding the utility of different classes of antihypertensive agents for reduction in cardiovascular mortality but confirm the importance of blood control as a general public health strategy (207, 209).

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CHAPTER 10

Acute Kidney Injury: Pathogenesis, Diagnosis, and Management

Charles L. Edelstein

Acute kidney injury (AKI) (defined as an increase in serum creatinine >0.5 mg/dL) occurs in 1% of hospital admissions (1), and up to 7% of hospitalized patients develop AKI (1). Twenty-five percent of patients in the intensive care unit (ICU) develop AKI as defined by oliguria or a serum creatinine >3.5 mg/dL (1). Five percent of patients in the ICU will need renal replacement therapy (RRT) (1,2). Dialysis is the only Federal Drug Administration (FDA)-approved treatment for AKI (3). Even though both intermittent hemodialysis (IHD) and continuous RRT (CRRT) are widely used, the reported mortality rates of AKI are between 30% and 80% (4,5). In spite of an increase in the degree of comorbidity of patients with AKI, the in-hospital mortality rate has declined over the period 1988 to 2002 (6).

AKI is defined as a sudden decrease in the glomerular filtration rate (GFR) occurring over a period of hours to days. The Acute Dialysis Quality Initiative (ADQI) has developed the RIFLE (Risk Injury, Failure, Loss of kidney function, and End-stage kidney disease) classification of AKI that divides AKI into the following stages: (a) risk, (b) injury, (c) failure, (d) loss of function, (e) and end-stage kidney disease (Fig. 10-1) (7–9). The term “acute kidney injury” replaces the term “acute renal

failure” (ARF), and ARF is restricted to patients who have AKI and need RRT. The RIFLE criteria have been validated in multiple studies, that is, as the RIFLE class increases, so does mortality (7–9).

The Acute Kidney Injury Network (AKIN) has also developed a classification of AKI (8–10) (Table 10-1). The AKIN group recommends a smaller change in serum creatinine (0.3 mg/dL) be used as a threshold to define the presence of AKI and identify patients with Stage 1 AKI (analogous to RIFLE-Risk). In the AKIN classification of AKI, a time period of 48 hours over which AKI occurs (compared to 1–7 days for the RIFLE criteria) is given. Patients receiving RRT are classified as Stage 3 AKI (RIFLE-Failure). In addition, the AKIN criteria differ from the RIFLE criteria as follows: (a) The AKIN classification includes less severe injury in the criteria. (b) AKIN avoids using the GFR as a marker in AKI, as there is no dependable way to measure GFR in AKI and equations to measure GFR in AKI are not reliable if the serum creatinine change is not in a steady state. (c) AKIN suggests that volume status should be optimized and urinary tract obstructions be excluded when using oliguria as a diagnostic criterion. The Kidney Disease Improving Global Outcomes (KDIGO) classification of AKI builds on the RIFLE and AKIN classifications. The KDIGO classification has both the increase in serum creatinine (0.3 mg/dL) over 48 hours and the 1.5- to 1.9-fold increase in serum creatinine known or presumed to have occurred over 1 to 7 days.

When AKI is not the result of primary vascular, glomerular, or interstitial disorders, it is referred to as acute tubular necrosis (ATN). In fact, in the clinical setting, the terms “acute renal failure” and “acute tubular necrosis” have become synonymous (11). However, ATN is a renal histologic finding and may not be consistently detectable in patients with AKI, despite profound kidney dysfunction (12–15). Thus, in the strictest sense, the terms AKI and ATN should not be used interchangeably (16). ATN has recently been defined as a syndrome of physiologic and pathologic dissociation (16).

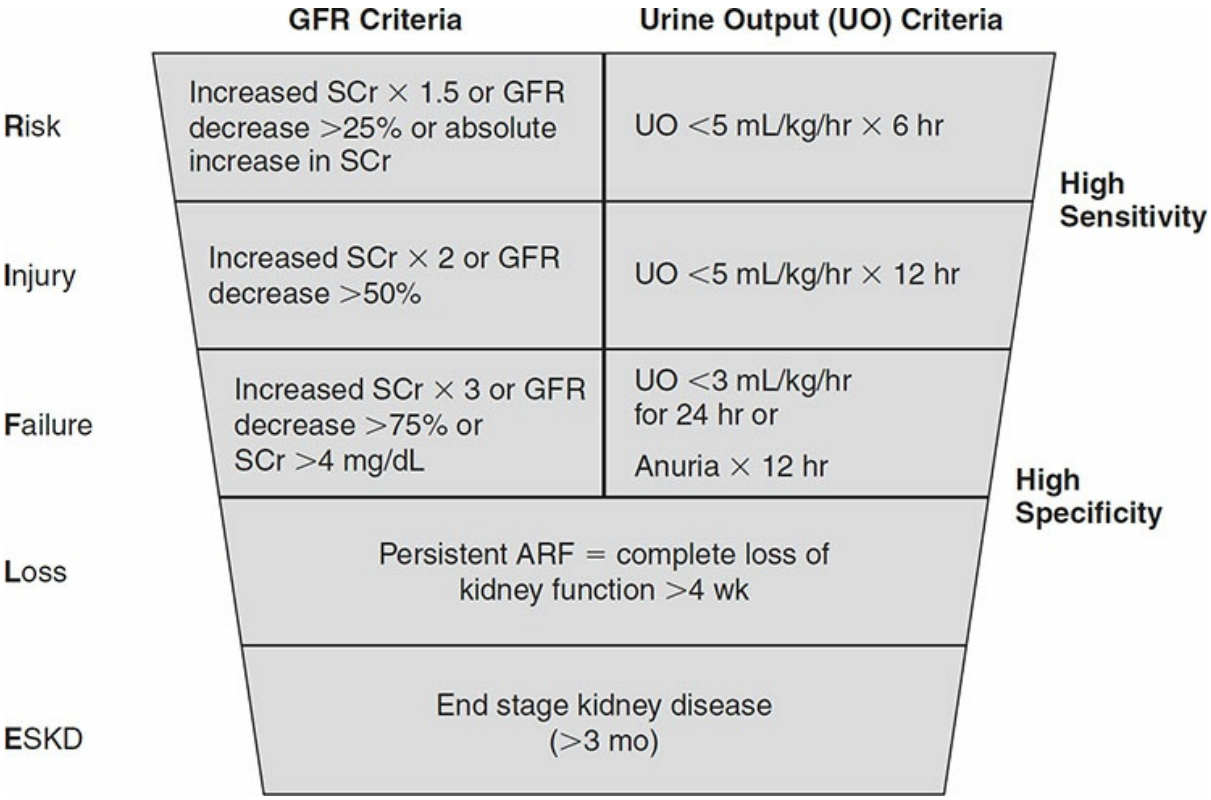

Figure 10–1 RIFLE criteria for the classification of AKI. RIFLE includes three grades of severity of AKI (risk, injury, and failure) and two outcome variables (loss of function and end-stage kidney disease). The RIFLE criteria attempt to convey the notion that kidney injury occurs before kidney failure. Studies have demonstrated that as the RIFLE class goes up, so does mortality. RIFLE, Risk, Injury, Failure, Loss of Kidney Function, and End-stage kidney disease; SCr, serum creatinine; AKI, acute kidney injury.

Table 10–1 AKIN and KDIGO Classification of AKI

Stage	Kidney Function	Urine Output
Stage 1	Increase in serum creatinine ≥0.3 mg/dL (within 48 h AKIN and KDIGO) or increase to ≥150%–199% (1.5- to 1.9-fold) from baseline (within 1–7 d KDIGO)	<0.5 mL/kg/h for ≥6 h
Stage 2	Increase in serum creatinine to 200%–299% (>2–2.9-fold) from baseline	<0.5 mL/kg/h for ≥12 h
Stage 3	Increase in serum creatinine to ≥300% (≥3-fold) from baseline or serum creatinine ≥4 mg/dL with an acute rise of at least 0.5 mg/dL or initiation of RRT	<0.3 mL/kg/h for ≥24 h or anuria for ≥12 h

The AKIN classification of AKI uses a smaller change in serum creatinine (0.3 mg/dL) to define the presence of AKI and identify patients with Stage 1 AKI

(analogous to RIFLE-Risk). The AKIN classification uses a time period of 48 hours over which AKI occurs (compared to 1–7 days for the RIFLE criteria). The KDIGO classification has both the increase in serum creatinine (0.3 mg/dL) over 48 hours and the 1.5 to 1.9-fold increase in serum creatinine known or presumed to have occurred over 1 to 7 days.
AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; KDIGO, Kidney Disease Improving Global Outcomes; RRT, renal replacement therapy.

Causes of AKI

INTRARENAL OR INTRINSIC AKI

After prerenal and postrenal azotemia have been excluded, the diagnosis of intrarenal or intrinsic AKI can be entertained. These problems may be renal vascular (large or small vessel), tubular, interstitial, or glomerular (Table 10-2). The Madrid AKI Study Group reported that the commonest cause of AKI was ATN accounting for 38% of hospitalized patients with AKI and 76% of ICU patients with AKI (4). The second and third leading causes of AKI were prerenal azotemia and urinary tract obstruction. Sepsis was the leading cause of AKI and more common than ischemic causes in the ICU (4,17–19). The diseases may be primary renal or part of a systemic disease. The diseases of vessels and glomeruli will be dealt with in Chapter 15. This chapter therefore will focus primarily on the ischemic and nephrotoxic causes of AKI and acute interstitial nephritis (AIN).

Table 10–2 Conditions That Cause "Intrinsic" or Parenchymal AKI

Vascular—Large Vessels
Bilateral renal artery stenosis
Bilateral renal vein thrombosis
Operative arterial cross clamping
Vascular—Small Vessels
Vasculitis
Atheroembolic disease
Thrombotic microangiopathies
Hemolytic uremic syndrome
Thrombotic thrombocytopenic purpura

Scleroderma renal crisis
Malignant hypertension
Hemolysis, elevated liver enzymes, and low platelet syndrome of pregnancy

Glomerular

In AKI, in the setting of glomerulonephritis, a rapidly progressive glomerulonephritis (RPGN) should be excluded. Extracapillary proliferation in the glomerulus forms crescents that can rapidly destroy the glomeruli.

Diseases with Linear Immune Complex Deposition

Goodpasture syndrome

Diseases with Granular Immune Complex Deposition

Acute postinfectious glomerulonephritis
Lupus nephritis
Infective endocarditis
Immunoglobulin A glomerulonephritis
Henoch–Schönlein purpura
Membranoproliferative glomerulonephritis
Cryoglobulinemia

Diseases with Few Immune Deposits ("Pauci-Immune")

Wegener granulomatosis
Polyarteritis nodosa
Idiopathic crescentic glomerulonephritis
Churg–Strauss syndrome

Interstitium

Acute allergic interstitial nephritis
Antibiotics
β-Lactam antibiotics (penicillins, methicillin, cephalosporins, rifampicin)
Sulfonamides
Erythromycin
Ciprofloxacin
Diuretics (furosemide, thiazides, chlorthalidone)
Nonsteroidal antiinflammatory drugs
Anticonvulsant drugs (phenytoin, carbamazepine)
Allopurinol
Interstitial nephritis associated with infection, granuloma, crystals
Streptococcal
Staphylococcal
Diphtheria
Leptospirosis

Brucellosis
Legionnaire's disease
Toxoplasmosis
Infectious mononucleosis
Salmonella typhi
Tuberculosis
Sarcoidosis
Acute uric acid nephropathy, e.g., tumor lysis syndrome
Hypercalcemia
Melamine toxicity

Acute Tubular Necrosis

Renal ischemia (50% of cases)
- Shock
- Complications of surgery
- Hemorrhage
- Trauma
- Gram-negative bacteremia
- Pancreatitis
- Pregnancy (postpartum hemorrhage, abruptio placenta, septic abortion)

Nephrotoxic drugs (35% of cases)
- Antibiotics (aminoglycosides, amphotericin, pentamidine, foscarnet, acyclovir)
- Antineoplastics (cisplatin, methotrexate)
- Iodine-containing x-ray contrast
- Organic solvents (carbon tetrachloride)
- Ethylene glycol (antifreeze)
- Anesthetics (enflurane)
- Acute phosphate nephropathy

Endogenous toxins
- Myoglobin due to rhabdomyolysis
- Hemoglobin (incompatible blood transfusion, acute falciparum malaria)
- Uric acid (acute uric acid nephropathy)

Pathogenesis of AKI

THE NATURE OF PROXIMAL TUBULAR INJURY

The nature of proximal tubular injury in ischemic AKI (20,21) includes reversible sublethal dysfunction (loss of polarity, swelling, loss of the apical brush border), lethal injury (necrosis necroptosis and apoptosis)

(13,20) and autophagy, a normal physiologic process that tries to rescue the destruction of cells in the body. Autophagy maintains homeostasis or normal functioning by protein degradation and turnover of the destroyed cell organelles for new cell formation.

In rat models of ischemic AKI and in posttransplant AKI in humans, there is reversible sublethal injury during the first 6 hours of reperfusion followed by necrosis at 24 hours of reperfusion (22–24). Proximal tubular cell death due to ischemic AKI in vivo in rodents and hypoxia in vitro results predominantly in necrosis, hence the term “acute tubular necrosis,” or ATN (25). Apoptotic cell death in ischemic renal injury in vivo has been demonstrated (26,27). When apoptosis has been demonstrated in early ischemic AKI, it is often present in the distal tubules (28–30). The significance of apoptosis in distal tubules is uncertain. Apoptosis in proximal tubules may play a role in tubular regeneration and was demonstrated to occur later at 3 days after ischemic injury in regenerating proximal tubules (31).

Dissociation of spectrin and other basolateral cytoskeletal proteins plays a major role in the well-documented sublethal injury and loss of polarity, which leads to proximal tubule dysfunction during renal ischemia (22,32,33). Spectrin is the major component of the membrane-associated cytoskeleton and is also important in the maintenance of cell membrane structural integrity. In the cytoskeleton of the proximal tubule, Na^+/K^+ ATPase is linked to the cytoskeleton/membrane complex by a variety of cytoskeletal proteins including spectrin (32,34). ATP depletion and renal ischemia cause dissociation of the basolateral cytoskeleton in rat kidneys (33,35) and in human transplanted kidneys (22). Na^+/K^+ ATPase and spectrin dissociate from the cytoskeleton during ischemic AKI (22).

A complete redistribution of Na^+/K^+ ATPase from the basolateral to the apical membrane, that is, total loss of polarity, is not necessary to decrease sodium reabsorption. It has been demonstrated that (a) translocation of Na^+/K^+ ATPase to the cytoplasm results in depolarization confined to the proximal tubule; (b) fractional excretion of lithium, a surrogate measure for the fraction of filtered sodium that is delivered to the macula densa, the site of tubuloglomerular feedback, is massively increased; and (c) these abnormalities persist for the duration of the maintenance phase of postischemic AKI (22,23). These results provide evidence for decreased proximal reabsorption of sodium, resultant increased sodium delivery to the macula densa, tubuloglomerular feedback (“tubular communication with the glomerulus”), and resultant filtration

failure that accompanies ischemic AKI.

The loss of polarity is also associated with redistribution of integrins. Tubular cells detach from their matrix, which results in increased cast formation and provides an experimental mechanism for the back-leak of glomerular filtrate. The consequences of loss of polarity, that is, tubuloglomerular feedback, cast formation with tubular obstruction, and back-leak of glomerular filtrate, are major factors in the pathogenesis of experimental ischemic AKI (26).

Necroptosis is a form of programmed or regulated necrosis or inflammatory cell death. Conventionally, necrosis is associated with unprogrammed cell death. Necroptosis shows that cells can execute necrosis in a programmed fashion and that apoptosis is not the only form of programmed cell death. Necroptosis is seen in human kidney cells subjected to ATP depletion (36). Necroptosis is mediated by receptor-interacting protein 1 (RIP1) and RIP3. Necroptosis was investigated in a mouse model of renal ischemia/reperfusion (I/R) injury. Treatment with necrostatin-1, an inhibitor of necroptosis, reduced organ damage and renal failure, even when administered after reperfusion (37,38). Inhibition of the core components of the necroptosis pathway RIP1 or RIP3 by gene knockout or a chemical inhibitor results in decreased cisplatin-induced proximal tubule damage in mice (39). Necroptosis is thought to contribute to AKI in kidney transplantation (40). The cytotoxicity of crystals of calcium oxalate, monosodium urate, calcium pyrophosphate dihydrate and cystine trigger caspase-independent necroptosis in five different cell types (41). These studies demonstrated that necroptosis is a major mechanism of proximal tubular cell death in AKI.

Autophagy is a process that takes place in all eukaryotic cells that keeps cells alive under stressful conditions (42). In autophagy, there is the sequestration of damaged organelles into double-membraned autophagosomes that subsequently fuse with lysosomes where their cargoes are delivered for degradation and recycling. In the healthy kidney, autophagy plays an important role in the homeostasis and viability of renal tubular epithelial cells.

Inhibition of autophagy using an ATG5 siRNA increases apoptosis during rewarming after cold storage in renal tubular epithelial cells (43). Autophagy occurs prior to apoptosis in renal tubular cells during AKI suggesting that autophagy is an early response of the cells to stress and not a result of apoptosis (44,45). Together, these studies suggest that autophagy is a renoprotective mechanism that protects against apoptosis to enable cell survival (42). Autophagy may be a protective mechanism to

decrease apoptosis through the degradation of mitochondria (46,47). Removal of mitochondria by autophagy can increase the threshold for induction of apoptosis (47). Depolarized mitochondria that are not cleared by autophagy release caspase activators (cytochrome c and Smac) into the cytoplasm to induce apoptosis.

In cisplatin-treated proximal tubule cells, inhibition of autophagy by pharmacological inhibitors or genetic knockdown increases apoptosis (44). In vitro, pharmacological or genetic suppression of autophagy sensitizes tubular cells to apoptosis induced by hypoxia (48). Mice with kidney-specific knockout of autophagy (ATG5 or ATG7) are viable but develop worse ischemic or cisplatin-induced AKI demonstrating the renoprotective role of autophagy in the kidney (44,45). Together, these studies suggest that autophagy is a renoprotective mechanism against apoptosis for cell survival (42).

Telomerase deficiency delays renal recovery in mice after I/R injury by impairing autophagy (49). Telomerase reverse transcriptase (TerT) and RNA (TerC) are essential to maintain telomere length. TerC or TerT knockout significantly delayed recovery in ischemic AKI. Electron microscopy and LC3-II showed a significant delay of autophagosome formation in TerC and TerT knockout mice. The mTORC1 inhibitor, rapamycin, partially restored the I/R-induced autophagy response.

In summary, basal autophagy in the kidney is vital for the normal homeostasis of the proximal tubules (50). There is a complex connection between autophagy, apoptosis, and regulated necrosis in AKI that merits further study (50).

Potential mediators/mechanisms of AKI cause tubular injury, inflammation, or vascular injury (Table 10-3). These mediators of tubular injury, inflammation, or vascular injury will now be discussed in more detail.

Tubular Injury

Ca^{2+} ACCUMULATION AND CELL INJURY

Ca^{2+} overload is characteristic of tissues with lethally injured cells, since the breakdown of the plasma membrane barrier to Ca^{2+} causes a large increase in cytosolic Ca^{2+}, which is sequestered in part by the mitochondria. Specifically, building on the hypothesis that homeostatic

mechanisms controlling cellular Ca^{2+} are disturbed in AKI, it has been shown that radiocontrast-induced AKI (51,52) and cadaveric kidney transplant dysfunction (53,54), for example, can be attenuated by administration of chemically dissimilar Ca^{2+} channel blockers. These are two clinical conditions in which intense renal vasoconstriction is demonstrable, a situation where delivery of oxygen and nutrients to renal tubules is compromised. The administration of Ca^{2+} channel blockers reduces the intensity of renal vasoconstriction and provides better delivery of nutrients to renal tissues. With ischemia, the poor nutrient flow to renal tubules also results in tubule Ca^{2+} overload, which can be lessened by the Ca^{2+} channel blockers. Although Ca^{2+} channel blockers have been shown to be efficacious in these two aforementioned clinical conditions, a full understanding of the mechanisms by which cytosolic or tissue Ca^{2+} increases in underperfused situations and how this increase may contribute to organ injury is the focus of much recent research. It is important, therefore, to understand the normal cellular Ca^{2+} regulation before discussing the newer insights that have been gained using experimental approaches to further improve our understanding of the pathogenesis of AKI.

Normal Regulation of Cell Ca^{2+}

Three major cellular Ca^{2+} pools exist: (a) a pool bound to plasma membranes, (b) a pool bound to or sequestered within intracellular organelles, and (c) a pool both free and bound within the cytoplasm (55).

Table 10–3 Mediators/Mechanisms of Ischemic AKI

Tubular Injury
Ca^{2+} influx (proximal tubules and afferent arterioles)
Disruption of actin cytoskeleton
Loss of polarity
Ca^{2+}-dependent PLA_2
Ca^{2+}-independent PLA_2
Calpain
Caspase-1
Caspase-3
Interleukin-18

Nitric oxide (generated by iNOS)
Metalloproteases
Defective heat shock response
Apoptosis
Regulated necrosis (RIP1, RIP3)
Defective autophagy
Altered gene expression
HIF-1α
Microparticles
miRNAs
Telomerase deficiency

Tubular Obstruction

Increased tubular pressure
Tamm–Horsfall protein
RGD peptides

Vascular Injury

Prostaglandins
Natriuretic peptides
Fractalkine
Abnormal vascular function
Increased sensitivity to vasoconstrictors
Increased sensitivity to renal nerve stimuli
Impaired autoregulation

Inflammation

Neutrophils
$CD4^+$ T cells
Macrophages
NK cells, NKT cells
Mast cells
Uric acid
Oxygen radicals
Endotoxin
Cytokines
Chemokines
Adhesion molecules
TLR4
HMGB1
NF-κB
IL-33, IL-17, IL-23
Inflammasome

AKI to CKD transition
Loss of peritubular microvessels
Epithelial-mesenchymal transition
TGF-βG2/M cell cycle arrest
PI3K, JNK, ERK, Akt
Endothelin
Selective epithelial injury
Cyr61

PLA_2, phospholipase A_2; HIF, hypoxia-inducible factor; NOS, inducible nitric oxide synthase; RGD, arginine–glycine–aspartic acid; NK, natural killer; NKT, natural killer T; TLR4, Toll-like receptor 4; HMGB1, high-mobility group box 1; NF-κB, nuclear factor-κB; IL, interleukin; AKI, acute kidney injury; CKD, chronic kidney disease; TGF, transforming growth factor; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase.

Although 60% to 70% of all Ca^{2+} in renal epithelial cells is located in the mitochondria, cytosolic free ionized Ca^{2+} is the most critical with regard to regulation of intracellular events. Cytosolic free Ca^{2+} $(Ca^{2+})i$ is normally kept at about 100 nM, which is 1/10,000 of the extracellular level (56). Ca^{2+} efflux is mediated on basolateral membranes by both Ca^{2+} ATPase, which is adenosine triphosphate (ATP) dependent, and a Na^+/Ca^{2+} exchanger on the basolateral membrane, which is ATP independent (57). Normally, the cell membrane is impermeable to Ca^{2+} and maintains a steep Ca^{2+} gradient between the cytosol and the extracellular space. However, when cytosolic Ca^{2+} increases in response to increased cellular membrane permeability or decreased Ca^{2+} efflux or both, the mitochondria and endoplasmic reticulum actively increase their Ca^{2+} uptake. Mitochondrial uptake and retention of Ca^{2+} become substantial only when cytosolic levels exceed 400 to 500 nM, as occurs with cell injury (56). Mitochondrial uptake is regulated by a Ca^{2+} uniporter in the mitochondrial inner membrane. During cell injury, active mitochondrial sequestration appears to be quantitatively the most important process for buffering elevations in cytosolic Ca^{2+}.

Tubular Effects of Ca^{2+} Accumulation

In vivo studies of intact kidney cannot discriminate between protective effects at the vascular sites compared to tubular sites or a combination

thereof. As the proximal tubule is the main site of injury in I/R models in vivo and the human allograft with AKI (58), the study of isolated proximal tubules during conditions of oxygen deprivation either in suspension or in primary culture has provided insight into the pathophysiology of proximal tubular injury. Numerous studies in both freshly isolated rabbit and rat proximal tubules as well as various models of proximal and distal tubules in culture have demonstrated an increase in cytosolic Ca^{2+} in these renal epithelial cells during chemical anoxia, hypoxia, and Ca^{2+} ionophore treatment (59–67). When exposed to anoxia in vitro, proximal and distal tubules in culture rapidly exhibit cell death after reoxygenation (68). However, if Ca^{2+} is removed from the bathing medium during the first 2 hours of reoxygenation and then replaced, cell viability is greatly enhanced (68). Ca^{2+} channel blockers have also been shown to delay the onset of anoxic cell death in primary cultures of rabbit proximal tubules and cortical collecting tubules, suggesting that Ca^{2+}-mediated hypoxic cell death is not limited to the proximal tubules (69).

Ca^{2+} channel blockers have no effects on the rate of Ca^{2+} influx into normoxic proximal tubules. However, during hypoxia or anoxia in vitro, Ca^{2+} influx rate into tubules is increased above normal levels, and Ca^{2+} channel blockers reduce this rate to or toward normal (70). This is an important observation because $(Ca^{2+})i$ could increase as the result of normal influx rates in the presence of reduced efflux rates secondary to decreased ATP-dependent Ca^{2+} ATPase or decreased Na^{+}/Ca^{2+} antiporter activity. The efficacy of Ca^{2+} channel blockers to prevent the increased Ca^{2+} influx rate during hypoxia and not during normoxia suggests a hypoxia-induced alteration in membrane permeability to Ca^{2+} that is sensitive to Ca^{2+} channel blockers. This permeability pathway appears to be sensitive, in part, to the decrease in ATP that occurs during hypoxia. For example, reduced ATP levels in rat proximal tubules with a phosphate-free incubation medium result in increased Ca^{2+} influx rate (71). This ATP-dependent change in Ca^{2+} permeability has not been examined in detail; however, acidosis prevents the increased Ca^{2+} influx rate in tubules and delays the onset of cell injury, as assessed by lactate dehydrogenase (LDH) release even though ATP remains at low levels (72). Cellular protection is also observed with an acidotic perfusate in the isolated perfused kidney (73). Intracellular acidosis is more likely to develop in complete anoxia than in hypoxia, and this may explain the only very short-lived increase in Ca^{2+} influx rate (70) as well as the absence of appreciable

tissue Ca^{2+} overload during anoxia, as assessed by atomic absorption spectroscopy (74).

On the basis of these observations, the role of Ca^{2+} influx rate in mediating proximal tubule hypoxic injury was examined. By employing a combination of ethylene glycol tetraacetic acid (EGTA) and various Ca^{2+} concentrations in the tubule bathing medium (Ca^{2+}-modified Krebs buffer), a delay in the onset of cell injury during hypoxia was seen when extracellular Ca^{2+} concentration was $<10^{-5}$ M (64).

Thus, Ca^{2+} ions enter renal proximal tubules at a faster rate than normal during oxygen deprivation. The removal of extracellular Ca^{2+} ions or administration of Ca^{2+} channel buffers reduces the injury associated with this increased influx rate of Ca^{2+}. Acidosis also reduces Ca^{2+} influx rate (72) and exerts cytoprotective effects (71–74). Finally, if Ca^{2+} ions do enter hypoxic or anoxic cells, their deleterious effects can be mitigated by calmodulin inhibitors (69). Together, these data strongly suggest that it is the increased cytosolic or intracellular burden of Ca^{2+} that initiates the development of cell injury.

The level of the free cytosolic Ca^{2+} increase during ATP depletion in proximal tubules has been studied. Previously it was difficult to determine peak cytosolic Ca^{2+} levels using the high-affinity Ca^{2+} fluorophore Fura-2. The $(Ca^{2+})i$ increases to >100 μM in ATP-depleted proximal tubules using the low-affinity Ca^{2+} fluorophore Mag-Fura-2 (75). Experiments were done in the presence of 2 mM glycine, which approximates the physiologic concentration in vivo. Ninety-one percent of the tubules studied in an individual experiment had a free cytosolic Ca^{2+} that exceeded 10 μM. Thirty-five percent had levels >500 μM with no cell membrane damage. In this study, proximal tubules had a remarkable resistance to the deleterious effects of increased Ca^{2+} during ATP depletion in the presence of glycine. In the isolated perfused rat kidney, intracellular Ca^{2+} increases have also been measured using 19^F NMR and 5^F BAPTA. In these studies, there was a partially reversible increase from 256 to 660 nM of Ca^{2+} (76,77).

The level of oxygen deprivation that is required to increase cytosolic Ca^{2+} has also been studied. A rise in cytosolic Ca^{2+} in anoxic but not hypoxic tubules was demonstrated (78). In hypoxic perfusion, oxygen tension measured with a very sensitive electrode was 5 to 6 mm Hg. Complete anoxia was achieved with oxyrase in a nonperfused system. Ca^{2+} did not increase during hypoxia, but there was an increase in Ca^{2+}

during anoxia. This increase paralleled the collapse in mitochondrial membrane potential as measured by rhodamine fluorescence. Because cell membrane damage occurred during both anoxia and hypoxia, it was concluded that an increase in cell Ca^{2+} is not always necessary for cell injury.

However, despite these studies, a crucial question remained to implicate Ca^{2+} as the primary factor in cell injury. Does the increase in cytosolic Ca^{2+} precede the injury, or is it a postlethal event? To answer this question, a video imaging system was designed in which the rise in cytosolic Ca^{2+} as well as cell membrane injury could be simultaneously measured in freshly isolated proximal tubules (79). $(Ca^{2+})i$ in freshly isolated proximal tubules, as assessed with Fura-2, increased significantly after 2 minutes of hypoxia and continued to increase progressively with continued hypoxia (67). This increase in $(Ca^{2+})i$ precedes the uptake by nuclei of the membrane-impermeable dye propidium iodide (PI) (67). PI staining is reduced when hypoxic rat proximal tubules are incubated either in a Ca^{2+}-free medium or with the intracellular Ca^{2+} chelator BAPTA (67). This study strongly supports the hypothesis that a cause-and-effect relationship exists between the elevation in $(Ca^{2+})i$ and the development of hypoxic membrane damage. Furthermore, this early rise in $(Ca^{2+})i$ after 5 to 10 minutes of hypoxia is reversible, since return to a well-oxygenated medium results in a prompt (1 minute) return of $(Ca^{2+})i$ to baseline level. If membrane injury had been the cause of the increase in $(Ca^{2+})i$, a return to basal levels would not have occurred with reoxygenation.

In support of a pathogenic role of Ca^{2+} in cell injury, it has been demonstrated that voltage-dependent Ca^{2+} channels are involved in cellular and mitochondrial accumulation of Ca^{2+} that follows ATP depletion and that voltage-dependent Ca^{2+} channels play an important role in regulating mitochondrial permeability transition, cytochrome c release, caspase activation, and apoptosis (80). In this study, in a rat renal proximal tubular cell line treated with antimycin A, ATP depletion-induced apoptosis was preceded by increased $[Ca(^{2+})]i$ and mitochondrial Ca^{2+} before activation of mitochondrial signaling. Antagonizing L-type $Ca(^{2+})$ channels with azelnidipine administration ameliorated cellular and mitochondrial $Ca(^{2+})$ accumulation, mitochondrial permeability transition, cytochrome c release, caspase-9 activation, and resultant apoptosis.

MECHANISMS OF Ca^{2+}-INDUCED PROXIMAL TUBULAR INJURY

There is now compelling evidence that hypoxia-induced rise in $(Ca^{2+})i$ activates Ca^{2+}-dependent intracellular events that mediate membrane injury. These potential Ca^{2+}-dependent mechanisms include changes in the actin cytoskeleton of proximal tubule microvilli, activation of phospholipase A_2 (PLA_2), and activation of the calcium-dependent cysteine protease, calpain.

Ca^{2+}-Dependent Changes in the Actin Cytoskeleton

In the presence of ATP depletion, both Ca^{2+}-independent as well as Ca^{2+}-mediated processes can disrupt the actin cytoskeleton during acute hypoxic proximal tubule cell injury (81,82). To better define the role of Ca^{2+} in pathophysiologic alterations of the proximal tubule microvillus actin cytoskeleton, freshly isolated tubules were studied. The intracellular free Ca^{2+} was equilibrated with highly buffered, precisely defined medium Ca^{2+} levels using a combination of the metabolic inhibitor, antimycin, and the ionophore, ionomycin, in the presence of glycine, to prevent lethal membrane damage (83). Increases in Ca^{2+} to ≥ 10 μM were sufficient to initiate concurrent actin depolymerization, fragmentation of F-actin into forms requiring high-speed centrifugation for recovery, redistribution of villin to sedimentable fractions, and structural microvillar damage consisting of severe swelling and fragmentation of actin cores. These observations implicate Ca^{2+}-dependent, villin-mediated actin cytoskeletal disruption in hypoxic tubule cell microvillar damage.

Ca^{2+}-Dependent Activation of PLA_2

PLA_2 hydrolyzes the acyl bond at the sn-2 position of phospholipids to generate free fatty acids and lysophospholipids. Free fatty acid release has been well documented in rat proximal tubules (84). This release is thought to be mediated to a large extent by activation of intracellular PLA_2 during hypoxia (85). It has been shown that both the messenger RNA (mRNA) for PLA_2 and the PLA_2 enzyme activity are increased in hypoxic rabbit tubules (86).

The mechanism of PLA_2-induced cell membrane damage is

controversial. In proximal tubules, hypoxia has been shown to cause an increase in free fatty acids, which was initially believed to contribute to cell injury (84). However, a recent study has shown that unsaturated free fatty acids protect against hypoxic injury in proximal tubules and that this protection may be mediated by negative feedback inhibition of PLA_2 activity (87).

There are various isoforms of PLA_2, and most isoforms of PLA_2 require Ca^{2+} for catalytic activity (88). The cytosolic form, $cPLA_2$, preferentially releases arachidonic acid from phospholipids and is regulated by changes in intracellular Ca^{2+} concentration (88).

PLA_2 enzymatic activity was measured in cell-free extracts prepared from rat renal proximal tubules (85). Both soluble and membrane-associated PLA_2 activity was detected. All PLA_2 activity detected during normoxia was Ca^{2+} dependent. Fractionation of cytosolic extracts by gel filtration revealed three peaks of PLA_2 activity. Exposure of tubules to hypoxia resulted in stable activation of soluble PLA_2 activity, which correlated with disappearance of the highest molecular mass form (>100 kDa) and appearance of a low-molecular-mass form (approximately 15 kDa) of PLA_2. Hypoxia also resulted in the release of a low-molecular-mass form of PLA_2 into the extracellular medium. This study provides direct evidence for Ca^{2+}-dependent PLA_2 activation during hypoxia. However, Ca^{2+}-independent forms of PLA_2 have also been found to play a role in hypoxic proximal tubular injury (89).

$cPLA_2$-deficient mice have been developed. The $cPLA_2$ knockout mice have smaller infarcts and develop less brain edema and fewer neurologic deficits after transient middle cerebral artery ischemia (90,91).

There is evidence of an increased macula densa cell calcium concentration with a reduction in fluid load to the macula densa (92). An increase in macula densa cell calcium activates PLA_2 to release arachidonic acid, the rate-limiting step in the formation of prostaglandins like PGE_2. Adenosine also has an important function in the juxtaglomerular apparatus. It stimulates calcium release in afferent arteriolar smooth muscle cells, leading to contraction of the afferent arteriole as part of the tubuloglomerular feedback mechanism.

CYSTEINE PROTEASES

The cysteine proteases are a group of intracellular proteases that have a cysteine residue at their active site. The cysteine proteases consist of three major groups: cathepsins, calpains, and caspases. The cathepsins are non–Ca^{2+}-dependent lysosomal proteases that do not appear to play a role in the initiation of lethal cell injury (93–95). Calpain is a cytosolic Ca^{2+}-activated neutral protease. The caspases are a family of intracellular cysteine proteases. The term "caspase" embodies two properties of these proteases in which "c" refers to "cysteine" and "aspase" refers to their specific ability to cleave substrates after an aspartate residue. Caspases play a crucial role in inflammation and apoptotic cell death.

Calpain

Calpain is a cytosolic neutral cysteine protease that has an absolute dependence on Ca^{2+} for its activation (96). There are two major ubiquitous or conventional isoforms of calpain, the low Ca^{2+}-sensitive μ-calpain and the high Ca^{2+}-sensitive μ-calpain (97,98). The isoenzymes have the same substrate specificity but differ in affinity for Ca^{2+}. Procalpain exists in the cytoplasm as an inactive proenzyme and becomes active proteolytically at the cell membrane only after it has become autolyzed (99,100). The autolyzed calpain is released either into the cytoplasm, where it hydrolyses substrate proteins, or it remains associated with the cell membrane and degrades cytoskeletal proteins involved in the interaction between the cell cytoskeleton and the plasma membrane. Activity of the autolyzed calpain is subject to a final regulation by a specific endogenous inhibitor called calpastatin (99,100). Calpain plays a role in platelet activation and aggregation (101), cytoskeleton and cell membrane organization (102,103), regulation of cell growth, differentiation, and development (104–106), and pathologic states, including Alzheimer disease, aging, cataract, muscular dystrophy, sepsis, Wiskott–Aldrich syndrome, Chédiak–Higashi syndrome, inflammation, arthritis, and malaria (107). Calpain 10 is a recently discovered mitochondrial calpain that plays a role in calcium-induced mitochondrial dysfunction (108).

The Ca^{2+}-dependent calpains have been shown to be mediators of hypoxic/ischemic injury to brain, liver, and heart (109–112). Calpain plays a role in hypoxic injury to rat renal proximal tubules (113–115). This role of calpain in proximal tubule injury has been confirmed in subsequent studies (116,117). The calpain inhibitors PD150606 and E-64 ameliorated the functional and histologic parameters in a rat model of ischemic AKI

(118). Injection of a fragment of calpastatin, which inhibits calpain, protects against the functional and histologic changes in the kidney in a mouse model of AKI (119). In recent studies, it has been demonstrated that calpains increase epithelial cell mobility and play a critical role in tubule repair. In vitro, exposure of human tubular epithelial cells (HK-2 cells) to μ-calpain reduced adhesion of HK-2 cells to extracellular matrix and increased their mobility. In a murine model of ischemic AKI, injection of a fragment of calpastatin, which specifically blocked calpain activity, delayed tubule repair and increased the worsening of kidney function and histologic lesions after 24 and 48 hours of reperfusion.

Caspases

Caspases are Ca^{2+}-independent cysteine proteases. There are 14 members of the caspase family, caspases 1 to 14. Caspase-14 has been characterized and found to be present in embryonic tissues but absent from adult tissues (120). Caspases share a predilection for cleavage of their substrates after an aspartate residue at P1 (121,122). The members of the caspase family can be divided into three subfamilies on the basis of substrate specificity and function (123). The peptide preferences and function within each group are remarkably similar (123). Members of group 1 (of which caspase-1 is the most important) prefer the tetrapeptide sequences Trp-Glu(OMe)-His-Asp(OMe) (WEHD) and YVAD =Ac-Tyr-Val-Ala-Asp (YVAD). This specificity is similar to the activation sequence of caspase-1, suggesting that caspase-1 may employ an autocatalytic mechanism of activation. Caspase-1 (previously known as interleukin-1 [IL-1] converting enzyme, or ICE) plays a major role in the activation of proinflammatory cytokines. Caspase-1 is remarkably specific for the precursors of IL-1 and IL-18 (interferon-γ-inducing factor), making a single initial cut in each procytokine that activates them and allows exit from the cytosol (124,125). Group III "initiator" caspase-8 and caspase-9 prefer the sequence (L/V)EXD. This recognition motif resembles activation sites within the "executioner" caspase proenzymes, implicating this group as upstream components in the proteolytic cascade that serve to amplify the death signal. These "initiator" caspases pronounce the death sentence. They are activated in response to signals indicating that the cell has been stressed or damaged or has received an order to die. They clip and activate another family of caspases, the "executioners." The optimal peptide sequence motif for group II, or "executioner caspases" (of which caspase-3 is the most important), is DEXD (123,126,127). This optimal recognition motif

is identical to proteins that are cleaved during cell death.

There are two major pathways of caspase-mediated apoptosis (128). In the mitochondrial or “intrinsic” pathway, stress-induced signals affect the balance between pro- and antiapoptotic Bcl-2 family proteins to cause cytochrome c release from mitochondria. Caspase-2 is a recently discovered caspase that is a crucial initiator of the mitochondrial apoptosis pathway (129). Activation and increased activity of caspase-2 is required for the permeabilization of mitochondria and release of cytochrome c (129). Cytochrome c binds to the cytosolic protein, apoptosis protease-activating factor-1 (APAF-1), which recruits and activates caspase-9. Active caspase-9 in turn recruits and activates the “executioners” procaspase-3 and procaspase-7. In the “extrinsic” pathway, the binding of a ligand to its death receptor recruits an adaptor protein that in turn recruits and activates procaspase-8. For example, Fas ligand (FasL) binds to its death receptor Fas that recruits an adaptor protein called Fas-associated death domain (FADD). FADD in turn recruits and activates procaspase-8.

The caspase pathways that are centrally important in cell death involve the “initiator” caspase-8 and caspase-9 and the “executioner” caspase-3 (130). The central role of these caspases is supported by caspase-8, caspase-9, and caspase-3 (−/−) mice that have strong phenotypes based on cell death defects, developmental defects, and usually fetal/perinatal mortality. The critical role of “initiator” caspases is illustrated in caspase-9 (−/−) mice that demonstrate the absence of downstream caspase-3 activation (131). Activation of caspase-1, caspase-8, caspase-9, and caspase-3 has been widely described in hypoxic renal epithelial cells and cerebral ischemia (28,132,133). Caspase-1 may also cause cell injury by activation of the proinflammatory cytokines IL-1 and IL-18 (125). To establish a direct pathogenic role of specific caspases in this well-established cascade, knockout mice have been used. Caspase-1 (−/−) mice are protected against cerebral ischemia (134). Caspase-3 (−/−) mice are protected against Fas-mediated fulminant hepatitis (135).

For many years it was not known how caspase-1 was activated. It has recently been discovered that procaspase-1 is activated in a complex called the inflammasome (136,137). The inflammasome is a protein scaffold that contains pyrin domain-containing protein (NALP) proteins, an adaptor protein apoptosis-associated speck-like protein containing a caspase-recruiting domain (CARD) (ASC), procaspase-1, and caspase-5. The interaction of the CARD of procaspase-1 is mediated by the CARD of ASC and the CARD present in the C-terminus of NALP-1. Active caspase-1 in the inflammasome is a regulator of the “unconventional” protein

secretion of “leaderless” proteins like IL-33, IL-1α, and fibroblast growth factor (FGF)-2 (138). IL-33 is an IL-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type-2 associated cytokines like IL-4, IL-5, and IL-13 that can lead to pathologic changes in mucosal organs (139). IL-1α is increased in the kidney in mice in endotoxemic AKI (140) and cisplatin-induced AKI (141).

As caspase-1 is activated in the inflammasome, we investigated the inflammasome in cisplatin-induced and ischemic AKI (142). To determine whether the NACHT, LRR and PYD domains (NLRP3) inflammasome plays an injurious role in cisplatin-induced AKI, we studied NLRP knockout NLRP3(−/−) mice. In cisplatin-induced AKI, the blood urea nitrogen (BUN), serum creatinine, ATN score, and tubular apoptosis score were not significantly decreased in NALP3(−/−) mice compared with wild-type mice. NLRP3(−/−) mice with ischemic AKI had significantly lower BUN, serum creatinine, and ATN and apoptosis scores than the wild-type controls. The difference in protection against cisplatin-induced AKI compared with ischemic AKI in NLRP3(−/−) mice was not explained by the differences in proinflammatory cytokines IL-1β, IL-6, chemokine (C-X-C motif) ligand 1, or tumor necrosis factor-α (TNF-α). Thus the NLRP3 inflammasome is a mediator of ischemic AKI but not cisplatin-induced AKI (142).

Caspases participate in two distinct signaling pathways, (a) activation of proinflammatory cytokines and (b) promotion of apoptotic cell death (121,127,143,144). While caspases play a crucial and extensively studied role in apoptosis, there is now considerable evidence that the caspase pathway may also be involved in necrotic cell death (145). Caspases and calpain are independent mediators of cisplatin-induced endothelial cell necrosis (146). Caspase inhibition has been demonstrated to reduce ischemic and excitotoxic neuronal damage (134,147,148). Moreover, mice deficient in caspase-1 demonstrate reduced ischemic brain injury produced by occlusion of the middle cerebral artery (133,134,149). Inhibition of caspases also protects against necrotic cell death induced by the mitochondrial inhibitor antimycin A in PC12 cells, Hep G2 cells, and renal tubules in culture (150,151). Caspases are also involved in hypoxic and reperfusion injury in cultured endothelial cells (152). Rat kidneys subjected to ischemia demonstrate an increase in both caspase-1 and caspase-3 mRNA and protein expression (25).

An assay for caspases in freshly isolated rat proximal tubules using the fluorescent substrate Ac-Tyr-Val-Ala-Asp-7-amido-4-methyl coumarin (Ac-YVAD-AMC) was developed (153). Freshly isolated proximal tubules

were preincubated with the caspase inhibitor Z-Asp-2, 6-dichlorobenzoyloxymethylketone (Z-D-DCB) for 10 minutes before being exposed to hypoxia. Tubular caspase activity was increased after 15-minute hypoxia in association with increased cell membrane damage as assessed by LDH release. Z-D-DCB attenuated the increase in caspase activity during 15-minute hypoxia and markedly decreased LDH release in a dose-dependent fashion. The fluorescent substrate Ac-DEVD-AMC, which is cleaved by caspase-3, was also used. Caspase activity was measured in normoxic and hypoxic tubules with both caspase-1 and caspase-3 substrates. Significant fluorescent activity was detected with Ac-YVAD-AMC (caspase-1 substrate) compared with Ac-DEVD-AMC (caspase-3 substrate), suggesting that caspase-1 is predominantly involved in hypoxic injury. In another study, the deleterious effect of caspase-1 on proximal tubules in vitro in the absence of inflammatory cells and vascular effects was demonstrated (154).

Caspase-1-Mediated Production of Interleukin-18

To establish a pathogenic role of caspase-1 in cell injury, caspase-1-deficient (−/−) mice have been used. These caspase-1 (−/−) mice have a defect in the production of mature IL-1β and IL-18 and are protected against lethal endotoxemia (149,155). The fact that IL-1β (−/−) mice are not protected against endotoxemia (156) suggests a potential role of IL-18 in the lethal outcome during sepsis. Moreover, in ischemic AKI, IL-1 receptor knockout mice or mice treated with IL-1 receptor antagonist (IL-1Ra) are not protected against ischemic AKI (157). Taken together, therefore, these previous studies suggest that IL-18 may be a potential mediator of ischemic AKI.

Since caspase-1 activates IL-18, lack of mature IL-18 might protect these caspase-1 (−/−) mice from AKI. Thus it was determined whether mice deficient in the proinflammatory caspase-1, which cleaves precursors of IL-1β and IL-18, were protected against ischemic AKI (158). Caspase-1 (−/−) mice developed less ischemic AKI as judged by renal function and renal histology. These animals had significantly reduced BUN and serum creatinine levels and a lower morphologic tubular necrosis score than did wild-type mice with ischemic AKI. In wild-type animals with ischemic AKI, kidney IL-18 levels more than double and there is a conversion of the IL-18 precursor to the mature form. This conversion was not observed in caspase-1 (−/−) AKI mice or sham-operated controls. Wild-type mice were then injected with IL-18-neutralizing antiserum before the ischemic insult,

and there was a similar degree of protection from AKI as seen in caspase-1 (−/−) mice. In addition, there was a fivefold increase in myeloperoxidase (MPO) activity, as an index of leukocyte infiltration, in control mice with AKI but no such increase in caspase-1 (−/−) or IL-18 antiserum-treated mice. Caspase-1 (−/−) mice also show decreased neutrophil infiltration, suggesting that the deleterious role of IL-18 in ischemic AKI may be due to increased neutrophil infiltration.

IL-18 function is neutralized in IL-18-binding protein transgenic (IL-18BP Tg) mice. It was determined whether IL-18BP Tg mice are protected against ischemic AKI (159). IL-18BP Tg mice were functionally and histologically protected against ischemic AKI, as determined by the BUN, serum creatinine, and ATN score. The number of macrophages was significantly reduced in IL-18BP Tg compared with wild-type kidneys. Multiple chemokines/cytokines were measured using flow cytometry-based assays. Only CXCL1 (also known as KC or IL-8) was significantly increased in AKI versus sham kidneys and significantly reduced in IL-18BP Tg AKI versus wild-type AKI kidneys. This study demonstrates that protection against ischemic AKI in IL-18BP Tg mice is associated with less macrophage infiltration and less production of CXCL1 in the kidney.

It was determined whether macrophages are a source of injurious IL-18 in ischemic AKI in mice (160). On immunofluorescence staining of the outer strip of the outer medulla, the number of macrophages staining for IL-18 was significantly increased in AKI and significantly decreased by macrophage depletion using tail vein injection of liposomal-encapsulated clodronate (LEC). Adoptive transfer of 264.7 cells, a mouse macrophage line that constitutively expresses IL-18 mRNA, or mouse peritoneal macrophages deficient in IL-18 reversed the functional protection against AKI in LEC-treated mice. In summary, adoptive transfer of RAW cells, that constitutively express IL-18, reverses the functional protection in macrophage-depleted wild-type mice with AKI. In addition, adoptive transfer of peritoneal macrophages in which IL-18 function was inhibited also reverses the functional protection in macrophage-depleted mice, suggesting that IL-18 from adoptive transfer of macrophages is not sufficient to cause ischemic AKI. Possible sources of injurious IL-18 in AKI include the proximal tubule and lymphocytes. In this regard, freshly isolated proximal tubules from mice release IL-18 into the medium when exposed to hypoxia, and proximal tubules from caspase-1-deficient mice are protected against hypoxic injury (154).

Caspase-1-deficient (−/−) mice are protected against sepsis-induced hypotension and mortality. The role of caspase-1 and its associated

cytokines was investigated in a nonhypotensive model of endotoxemic AKI. In mice with endotoxemic AKI, the GFR was significantly higher in caspase-1 (−/−) versus wild-type mice at 16 and 36 hours. IL-1β and IL-18 protein were significantly increased in the kidneys of mice with endotoxemic AKI versus vehicle-treated mice. However, inhibition of IL-1β with IL-1Ra, or inhibition of IL-18 with IL-18-neutralizing antiserum-treated or combination therapy with IL-1Ra plus IL-18-neutralizing antiserum did not improve the GFR in mice with endotoxemic AKI, suggesting that neither IL-1β nor IL-18 is the mediator on endotoxemic AKI (140).

The role of IL-18 was investigated in cisplatin-induced AKI. In IL-18Rα knockout vs. wild-type mice with cisplatin-induced AKI, there was worse kidney function, tubular damage, increased accumulation of $CD4^+$ and $CD8^+$ T cells, macrophages, and neutrophils, upregulation of early kidney injury biomarkers (serum TNF, urinary IL-18, and KIM-1 levels), and increased expression of proinflammatory molecules downstream of IL-18 (161). Anti-IL-18Rα and anti-IL-18Rβ antibody treatment increased cisplatin nephrotoxicity in wild-type mice. Thus, signaling through the IL-18 receptor α attenuates inflammation in cisplatin-induced AKI (161). Cisplatin-induced AKI is associated with an increase in cytokines including IL-18 in the kidney (141). However, IL-18 antiserum or transgenic mice that overexpress IL-18 binding protein, a natural inhibitor of IL-18, were not protected against cisplatin-induced AKI (141). Thus, unlike ischemic AKI where IL-18 is a mediator of injury, IL-18 is not a mediator of cisplatin-induced AKI.

Interaction between Calpain and Caspases in Hypoxic/Ischemic Proximal Tubular Injury

Studies suggest that both calpain and caspases play a role in hypoxia-induced cell membrane damage in proximal tubules (25,113,115,150,153). A prelethal increase in cytosolic Ca^{2+} is a cardinal feature of the hypoxic proximal tubule model (67). How are the non–Ca^{2+}-dependent caspases activated during hypoxia? There are two possibilities. Caspase activation may be downstream of Ca^{2+}-mediated activation of calpain, or caspases may be activated in a separate pathway independent of Ca^{2+}. Since an interaction between caspases and calpains during cell injury has been suggested (149), the effect of the specific calpain inhibitor (2)-3-(4-iodophenyl)-2-mercapto-2-propenoic acid (PD150606) on the hypoxia-

induced increase in caspase activity in proximal tubules was studied (153). PD150606 inhibited calpain activity and protects against hypoxic injury in rat proximal tubules (114). PD150606 also attenuated the hypoxia-induced increase in caspase activity. However, PD150606 did not inhibit the activity of purified caspase-1 in vitro, suggesting that calpain may be upstream of caspases during hypoxic proximal tubular injury. Next, the effect of caspase inhibition on calpain activity was determined (153). The specific caspase inhibitor Z-D-DCB attenuated the hypoxia-induced increase in calpain activity in proximal tubules. However, Z-D-DCB did not inhibit the activity of purified calpain in vitro.

In summary, these data suggest that both caspase-mediated activation of calpain and calpain-mediated activation of caspases occur during hypoxic proximal tubular injury. These data are supported by other studies that demonstrate simultaneous activation of both calpain and caspases during cell death (162). Thus, it is possible that during hypoxic proximal tubule injury, there are different proteolytic pathways involving different caspases and calpains.

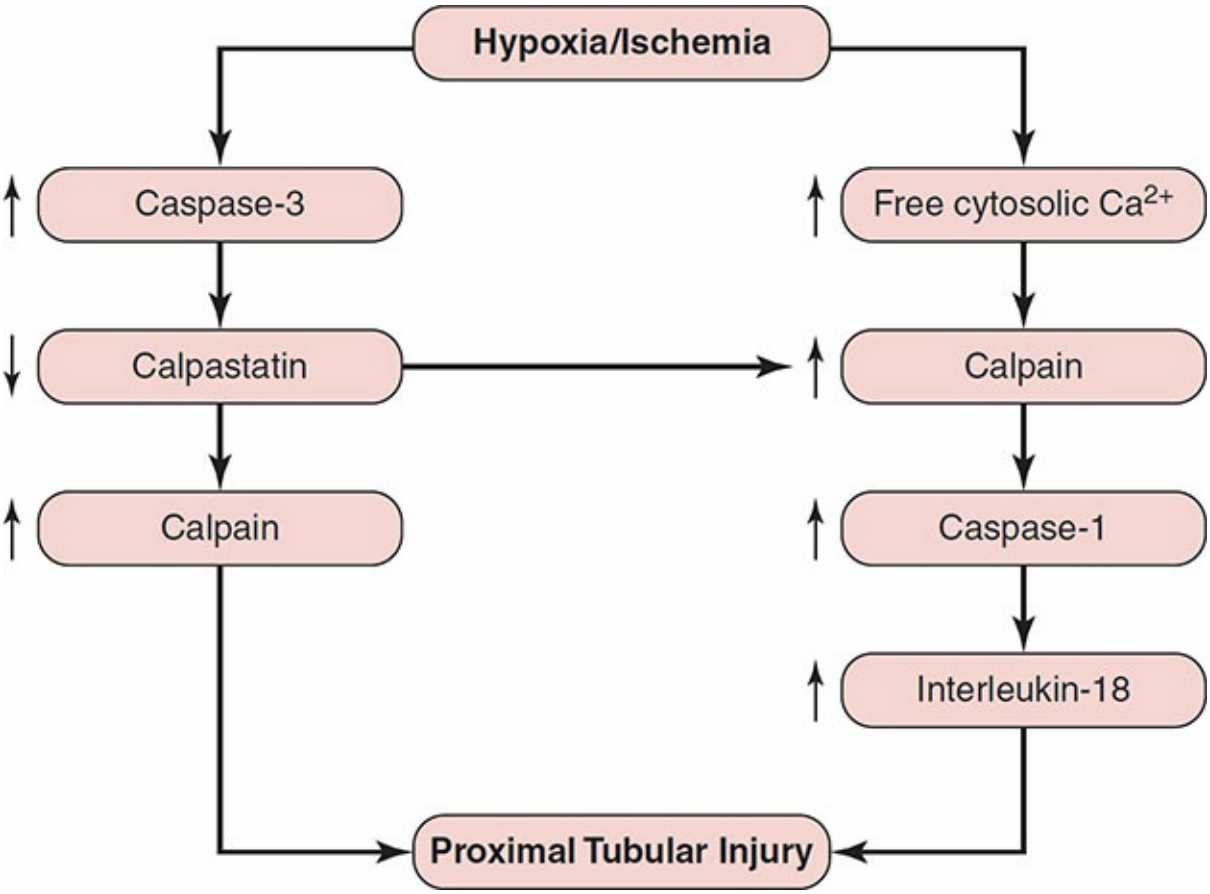

Figure 10–2 Calpains and caspases in proximal tubular necrosis. Hypoxic/ischemic proximal tubular necrosis results in activation of cysteine protease pathways involving calpains and both caspase-1 and caspase-3 (164). There is increased activity of calpain

(113–115) and caspase-1 (153) in hypoxic proximal tubular injury. During ischemic AKI, there is early calpain activation associated with downregulation of calpastatin protein, decreased calpastatin activity, and activation of caspase-3 (163). Also, impaired IL-18 processing protects caspase-1-deficient mice from ischemic AKI (158).

The interaction between calpain and caspases during ischemic AKI in vivo was investigated (163). An increase in the activity of calpain, as determined by (a) the appearance of calpain-mediated spectrin breakdown products and (b) the conversion of procalpain to active calpain, was demonstrated. Since intracellular calpain activity is regulated by its endogenous inhibitor, calpastatin, the effect of ischemia on calpastatin was determined. On immunoblot of renal cortex, there was a decrease of a low-molecular-weight form of calpastatin during ischemic AKI compared to sham-operated controls. Calpastatin activity was also significantly decreased compared to sham-operated rats, indicating that the decreased protein expression had functional significance. In rats treated with the caspase inhibitor Z-D-DCB, the decrease in both calpastatin activity and protein expression was normalized, suggesting that caspases may be proteolyzing calpastatin. Caspase-3 activity increased significantly after I/R compared to sham-operated rats and was attenuated in ischemic kidneys from rats treated with the caspase inhibitor. In summary, during ischemic AKI there is (a) calpain activation associated with downregulation of calpastatin protein and decreased calpastatin activity and (b) activation of caspase-3. In addition, in vivo caspase inhibition reverses the decrease in calpastatin activity. The proposed relationship between calpain and caspases in hypoxic/ischemic injury is shown in Figure 10-2 (153,158,164).

ROLE OF NITRIC OXIDE IN HYPOXIA/ISCHEMIA-INDUCED PROXIMAL TUBULE INJURY

Nitric oxide (NO) is a messenger molecule mediating diverse functions, including vasodilatation, neurotransmission, and antimicrobial and antitumor activities (165). A variety of cells produce NO via oxidation of L-arginine by the enzyme nitric oxide synthase (NOS) (166). Thus far, four distinct NOS isoforms have been isolated, purified, and cloned: neuronal, endothelial, macrophage, and vascular smooth muscle cell (VSMC)/hepatocyte (167,168). Identification of the specific isoform of NOS is important because the four isoforms vary in subcellular location, amino acid sequence, regulation, and hence functional roles. Neuronal and endothelial NOS (eNOS) are continuously present and thus are termed

constitutive NOS (cNOS) (168). NO is produced by these enzymes when Ca^{2+}/calmodulin interaction permits electron transfer from nicotinamide adenine dinucleotide phosphate (NADPH) via flavin groups within the enzyme to a heme-containing active site (169). This activation is very short lived. In contrast, VSMC/hepatocyte and macrophage isoforms are only expressed when the cells have been induced by certain cytokines, microbes, and microbial products and are therefore called inducible NOS (iNOS) (170). iNOS expression results in sustained production of NO. Unlike cNOS, iNOS activity is believed to be insensitive to changes in intracellular Ca^{2+}, since calmodulin is tightly bound to the molecule. Once synthesized, iNOS remains tonically activated, producing NO continuously for the life of the enzyme (171).

Both cNOS and iNOS isoforms have been identified in the kidney, specifically in macula densa cells (cNOS), inner medullary collecting ducts (cNOS and iNOS), and proximal tubules (cNOS and iNOS) (168,172). In the kidney, physiologic amounts of NO play an important role in hemodynamic regulation and salt and water excretion (173).

It has been demonstrated that NOS activity is increased during hypoxia in freshly isolated rat proximal tubules. In this study, membrane damage, as assessed by LDH release into the medium, was prevented by both a nonselective NOS inhibitor (L-NAME) and a NO scavenger (hemoglobin) (174). In a separate study, hypoxia stimulated prompt and sustained NO release in the proximal tubule suspension as assessed by a NO-selective sensing electrode (175). NO concentration remained unmeasurable during normoxia. L-NAME completely inhibited hypoxia-induced NO release in parallel with marked cytoprotection. Further studies in freshly isolated proximal tubules from knockout mice have also been revealing about the role of NO in hypoxic/ischemic tubular injury. Hypoxia-induced proximal tubule damage, as assessed by LDH release, was no different between wild-type mice in which eNOS and nNOS were “knocked out.” However, proximal tubules from the iNOS knockout mice demonstrated resistance to the same degree of hypoxia (176).

In vivo, targeting of iNOS with oligodeoxynucleotides protects the rat kidney against ischemic AKI (177). This study provided direct evidence for the cytotoxic effects of NO produced via iNOS in the course of ischemic AKI. Augmented expression of iNOS and the prevalence of nitrotyrosine residues in kidneys have been demonstrated in osteopontin-deficient mice versus wild-type counterparts (178). Animals with the disrupted osteopontin gene exhibited ischemia-induced renal dysfunction and structural damage, which was twice as pronounced as that observed in

mice with the intact osteopontin response to stress, also suggesting a role of iNOS in ischemic AKI. iNOS-deficient mice also have less renal failure and better survival than the wild-type mice after renal artery clamping (179). An induction of heat shock protein (HSP) was also observed in the iNOS knockout mice as a potential contributor to the protection.

In a renal artery clamp model in mice in which alpha-melanocyte-stimulating hormone (*ά*-MSH) was shown to block the induction of iNOS, there was decreased neutrophil infiltration and functional and histologic protection (180). A subsequent study examined the relative importance of *ά*-MSH on the neutrophil pathway by examining the effects of *ά*-MSH in ICAM-1 knockout mice and the neutrophil-independent isolated perfused kidneys (181,182). In this study, it was found that *ά*-MSH decreases renal injury when neutrophil effects are minimal or absent, indicating that *ά*-MSH inhibits neutrophil-independent pathways of renal injury.

Interestingly, however, L-NAME administration to the rat kidney clamp model actually worsens ischemic and endotoxemic AKI (177). This result was interpreted as an overriding blocking effect of eNOS activity with the nonspecific effects of L-NAME (26). This would worsen the renal vasoconstriction and resultant injury, thus obscuring any salutary effect at the level of the proximal tubule (183). Thus, opposing abnormalities in NO production within the endothelial and tubular compartments of the kidney may contribute to renal injury (26) (Fig. 10-3). Reduced eNOS-derived NO production causes vasoconstriction and worsens ischemia; increased iNOS-derived NO production by tubular cells adds to the injurious effects of ischemia on these cells. Therapeutic interventions to modulate NO production in ischemic AKI may require selective modulation of different NOS isoforms in the tubular and vascular compartments of the kidney (184).

MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMP) play a crucial role in remodeling of the extracellular matrix, which is an important physiologic feature of normal growth and development. In the kidney, interstitial sclerosis and glomerulosclerosis have been associated with an imbalance of extracellular matrix synthesis and degradation (185). Alterations in renal tubular basement membrane matrix proteins, laminin and fibronectin, occur after renal I/R injury (186).

Meprin A is a zinc-dependent metalloendopeptidase that is present in the brush border membrane of renal proximal tubular epithelial cells. The

redistribution of this metalloendopeptidase to the basolateral membrane domain during AKI results in degradation of the extracellular matrix and damage to adjacent peritubular structures. The effect of meprin A, the major matrix-degrading metalloproteinase in rat kidney, on the laminin–nidogen complex was examined. Following ischemic injury, meprin A undergoes redistribution and/or adherence to the tubular basement membrane. Nidogen-1 (entactin), which acts as a bridge between the extracellular matrix molecules, laminin-1 and type IV collagen breakdown products, is produced as the result of partial degradation of tubular basement membrane by meprin A following renal tubular I/R injury (187).

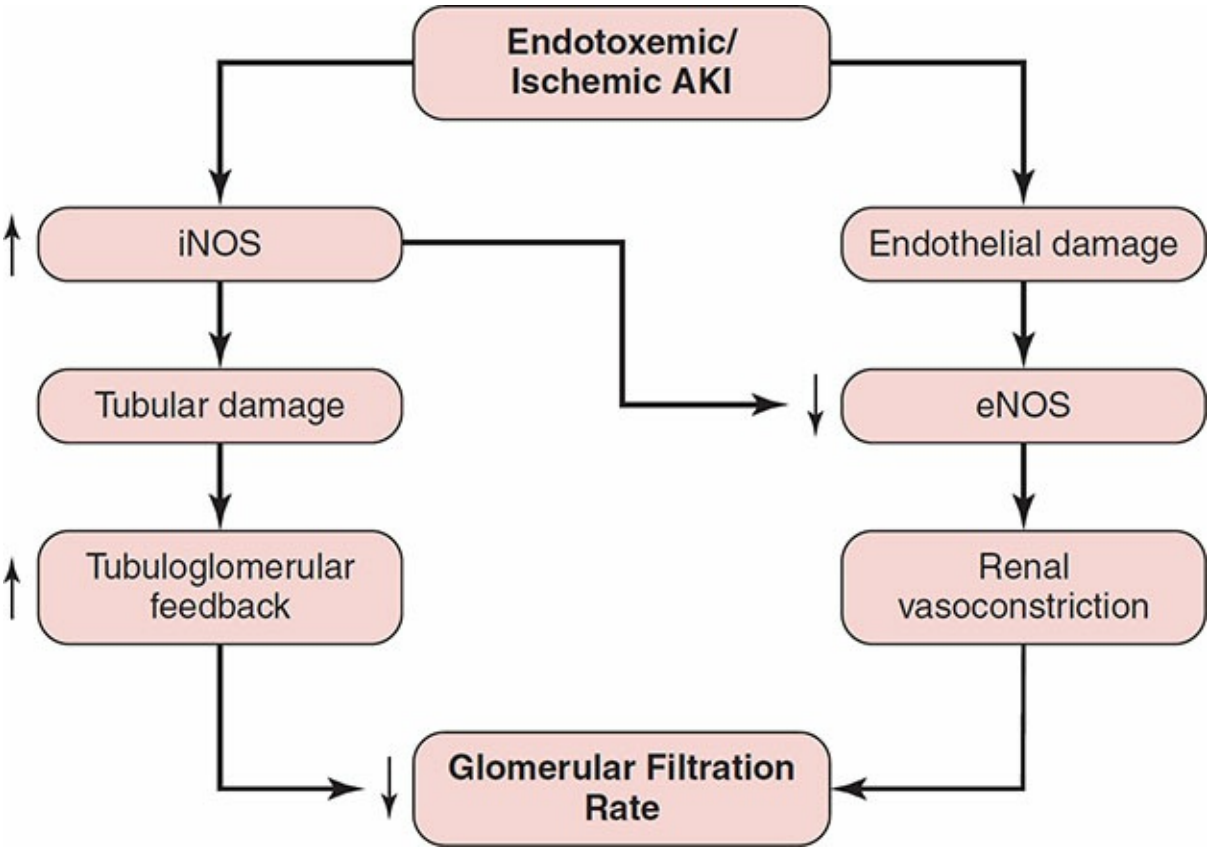

Figure 10–3 Proposed imbalance of NO production in ischemic/septic AKI. In ischemic AKI, increased NO derived from iNOS is damaging to proximal tubules (176,177,179). In ischemic AKI, renal endothelial damage results in decreased NO derived from eNOS (26). In endotoxemic AKI, increased iNOS activity decreases eNOS activity possibly via NO autoinhibition (387). The nonselective NOS inhibitor, L-NAME, worsens ischemic and endotoxemic AKI due to an overriding blocking effect on eNOS.

Inbred strains of mice with normal and low meprin A activity have been studied (188). The strains of mice with normal meprin A developed more severe renal functional and structural injury following renal ischemia

or the injection of hypertonic glycerol compared with the two low-meprin A strains. These findings suggest that meprin A plays a role in the pathophysiology of AKI following ischemic and nephrotoxic AKI (188).

The disruption of cadherin/catenin complexes in AKI may be associated with the transtubular back-leak of glomerular filtrate. In endothelial cells isolated from ischemic kidneys, the proteolytic activity of proMMP-2, proMMP-9, and MMP-9 was increased. Occludin, an in vivo MMP-9 substrate, was partly degraded in the endothelial fractions during ischemia, suggesting that the upregulation of MMP-9 was functional. These data suggest that AKI leads to the degradation of the vascular basement membrane and to increased permeability related to the increase in MMP-9 (189). In renal cells, in vitro cleavage of cadherins in normal rat kidney (NRK) cells requires active membrane-type (MT)1-MMP (MT1-MMP), also known as MMP-14 (190). In contrast to the potential injurious role of some MMPs, MMP9 protects the S3 segment of the proximal tubule and the intercalated cells of the collecting duct from apoptosis in AKI, most likely by releasing soluble stem cell factor, an MMP9 substrate (191).

HEAT SHOCK PROTEINS

HSPs protect cells from environmental stress damage by binding to partially denatured proteins and dissociating protein aggregates to regulate the correct folding and to cooperate in transporting newly synthesized polypeptides to the target organelles (192). Stresses that trigger the heat shock response include hyperthermia, hypothermia, generation of oxygen radicals, hypoxia/ischemia, and toxins (193).

HSPs are identified by their molecular weight. The most important families include proteins of 90, 70, 60, and 27 kDa (193). The HSP70 family includes proteins that are both constitutively expressed and induced by stress. They are the most highly induced proteins by stress and function as chaperones binding to unfolded or misfolded proteins.

Renal ischemia results in both a profound fall in cellular ATP and a rapid induction of HSP70 (194,195). It has been demonstrated that a 50% reduction in cellular ATP in the renal cortex during ischemia must occur before the stress response is detectable. Reduction in ATP below 25% control levels produces a more vigorous response. Reperfusion is not required for initiation of a heat shock response in the kidney (196).

In vitro studies have demonstrated that HSP induction protects cultured renal epithelial cells from injury. It has been determined that prior heat

stress protects cultured opossum kidney (OK) cells from injury mediated by ATP depletion (197). Also HSP70 overexpression is sufficient to protect LLC-PK1 proximal tubular cells from hyperthermia but is not sufficient for protection from hypoxia (198).

The effect of HSP induction by hyperthermia on ischemic AKI has been studied. One study found that prior heat shock protected kidneys against warm ischemia (199). In another study, prior induction of HSP by hyperthermia was not protective against the functional and morphologic parameters of ischemic AKI in ischemia reflow in intact rats or medullary hypoxic injury (200). These variable results may be explained by the complexity of the intact animal compared with cultured cells; the degree, duration, and timing of the hyperthermic stimulus; and the differential response of mature and immature kidneys (201,202).

The mechanism of HSP protection against ischemic AKI is evolving. It has been demonstrated that HSPs participate in the postischemic restructuring of the cytoskeleton of proximal tubules (203). HSP72 complexes with aggregated cellular proteins in an ATP-dependent manner, suggesting that enhancing HSP72 function after ischemic renal injury assists refolding and stabilization of Na^+/K^+ ATPase or aggregated elements of the cytoskeleton, allowing reassembly into a more organized state (204). Another study suggested that there are specific interactions between HSP25 and actin during the early postischemic reorganization of the cytoskeleton (205).

Another potential mechanism of HSP protection against proximal tubular injury is the inhibition of apoptosis. OK proximal tubule cells exposed to ATP depletion develop apoptosis, and prior heat stress reduced the number of apoptotic cells and improved cell survival compared with controls (206).

APOPTOSIS

Apoptosis is a physiologic form of cell death that occurs in a programmed pattern and can be triggered by external stimuli (207). The triggers of apoptosis include (a) cell injury, for example, ischemia, hypoxia, oxidant injury, NO, and cisplatinum; (b) loss of survival factors, for example, deficiency of renal growth factors, impaired cell-to-cell or cell-to-matrix adhesion; and (c) receptor-mediated apoptosis, for example, Fas (CD 95) and transforming growth factor-β (TGF-β) (208).

Apoptosis has been demonstrated in cultured proximal and distal tubules exposed to hypoxia and chemical ATP depletion

(132,206,207–211). A feature of these in vitro studies is that severe or prolonged ATP depletion leads to necrosis, while milder and shorter ATP depletion leads to apoptotic cell death (132). Apoptosis has been demonstrated in distal and proximal tubules during both the early phase and the recovery phase of ischemic AKI in rats and mice (28–30,212–222). The role that apoptosis of proximal and distal tubular cells plays in the loss of renal function and the recovery phase of ischemic AKI, as well as the relationship between apoptosis and necrosis in ischemic AKI, still needs to be elucidated (208,223,224).

Cisplatin is a commonly used chemotherapeutic agent that causes apoptosis or necrosis of renal tubular epithelial cells in vitro. After cisplatin injection in mice, renal apoptosis peaks on day 2, which precedes the peak in serum creatinine, ATN scores, and neutrophil counts, which peak on day 3. Renal dysfunction, apoptosis, ATN scores, and neutrophil infiltration were all reduced in caspase-1 (−/−) mice treated with cisplatin. Active caspase-3 was also reduced in caspase-1 (−/−) mice (225). This study confirms the injurious role of caspases and apoptosis in cisplatin-induced AKI.

Erythropoietin (EPO) is upregulated by hypoxia. EPO receptors are expressed in many tissues, including renal tubules. Multiple animal studies have shown that EPO is protective against AKI, and the protective effect may be related to inhibition of caspases and apoptosis (Table 10-4). In a cisplatin-induced AKI model in the rat, functional recovery was significantly improved in animals that received EPO compared with controls, and the enhanced recovery was secondary to increased regeneration of tubules, as shown by increased uptake of radioactive thymidine (226). In another study, rats that were pretreated with EPO before induction of ischemic AKI had a lower serum creatinine and decreased apoptosis compared with controls (227). In both in vivo and in vitro models of tubular injury, EPO provided protection from I/R injury by inhibiting apoptosis and increasing tubular cell regeneration (228). EPO was shown to be protective against interstitial fibrosis and inflammation in a rat model of cyclosporine nephrotoxicity (229). EPO prevents the decrease in the GFR in a rat model of contrast nephropathy (230). Kolyada et al. demonstrated that EPO decreased iohexol-induced activation of caspase-3 and caspase-8 and subsequent apoptosis in renal tubular epithelial cells (231). EPO and/or α-MSH treatment significantly prevented urinary-concentrating defects and downregulation of renal aquaporins (AQP) and sodium transporters in ischemic AKI in rats (232). EPO (300 units/kg) reduced tubular injury, prevented caspase-3, caspase-

8, and caspase-9 activation, and reduced apoptotic cell death in vivo in mice (233). In human proximal tubule epithelial cells in vitro, EPO reduced DNA fragmentation, prevented caspase-3 activation, and attenuated cell death in response to oxidative stress (233). In a rat model of hemorrhagic shock, administration of EPO before resuscitation reduced the increase in the activities of caspase-3, caspase-8, and caspase-9, and prevented renal dysfunction and liver injury (234). In a model of endotoxemia-induced AKI in mice, EPO significantly decreased renal superoxide dismutase and attenuated the renal dysfunction as assessed by insulin-GFR (235).

Table 10–4 Erythropoietin Protects against AKI

Model	Mechanism	References
Cisplatin-induced AKI in rats	Increased tubular regeneration	(226)
Ischemic AKI in rats	Functional protection Less apoptosis	(227) —
Ischemic AKI in rats	Decreased apoptosis Increased tubular cell regeneration	(228) —
Rat AKI	Functional protection Decreased caspase-3, caspase-8, and caspase-9 Less apoptosis	(233) — —
Proximal tubules exposed to oxidative stress	Decreased caspase-3 and cell death	(233)
Tubular cells exposed to iohexol	Decreased activation of caspase-3 and caspase-8 Less apoptosis	(231) —
Hemorrhagic shock in rats	Less AKI Less liver injury Decreased caspase-3, caspase-8, and caspase-9	(234) — —
Ischemic AKI in rats	Prevented downregulation of renal sodium transporters and aquaporins	(232)
Cyclosporin nephrotoxicity in	Less inflammation	(229)

rats	Less interstitial fibrosis	—
Contrast nephropathy in rats	Less AKI	(230)
Endotoxemic AKI in mice	Decreased superoxide dismutase Less renal dysfunction	(235) —

AKI, acute kidney injury.

The β-common receptor (βcR) plays an important role in the nonhematopoietic tissue-protective effects of EPO. In a mouse model of lipopolysaccharide (LPS)-induced AKI, the AKI was attenuated by EPO given 1 hour after LPS in wild-type but not in βcR knockout mice (236). In a cecal ligation model of AKI in older mice, AKI was attenuated by EPO treatment in wild-type mice but not in βcR knockout mice. Thus, activation of the βcR by EPO is essential for the protection against AKI in either endotoxemic young mice or older mice with polymicrobial sepsis, and for the activation of well-known signaling pathways by EPO (236).

Elimination of the mitochondrial fusion protein mitofusin 2 (Mfn2) sensitizes proximal tubular cells to apoptosis in vitro (237). The role of proximal tubular Mfn2 in ischemic AKI in vivo was investigated in ischemic AKI. Mice with a conditional knock out of proximal tubular Mfn2 (cKO-PT-Mfn2) had much less survival than wild-type mice with ischemic AKI. Increased cell proliferation, but no significant differences in ATN score, apoptosis, or necrosis were detected between genotypes. In ATP depletion in vitro, Mfn2 deficiency significantly increased proximal tubular proliferation and persistently activated extracellular signal-regulated kinase 1/2 (ERK1/2). Ischemic AKI reduced the Mfn2-Retrovirus-associated DNA sequences (RAS) interaction and increased both RAS and p-ERK1/2 activity in the renal cortical homogenates of cKO-PT-Mfn2 mice. These results suggest that, in contrast to its proapoptotic effects in vitro, selective PT Mfn2 deficiency accelerates recovery of renal function and enhances animal survival after ischemic AKI in vivo, in part by increasing Ras-ERK-mediated cell proliferation.

Conformational change in transfer RNA is an early indicator of acute cellular damage before the detection of apoptosis (238) Using a tRNA-specific modified nucleoside 1-methyladenosine (m1A) antibody, it was demonstrated that oxidative stress induces a direct conformational change in tRNA structure that promotes subsequent tRNA fragmentation and occurs much earlier than DNA damage. In various models of tissue

damage (ischemic reperfusion, toxic injury, and irradiation), the levels of circulating tRNA derivatives increased rapidly. In humans, the levels of circulating tRNA derivatives also increased early in ischemic AKI before other known tissue injury biomarkers. It was concluded that tRNA damage reflects early oxidative stress damage, and detection of tRNA damage may be a useful tool for identifying organ damage (238).

ALTERED GENE EXPRESSION

Immediate early genes and protooncogenes are induced during the early reperfusion period after renal ischemia (239). There is c-fos and c-jun activation as well as an increase in DNA synthesis (240). There is accumulation of early growth response factor-1 (Egr-1) and c-fos mRNAs in the mouse kidney after occlusion of the renal artery and reperfusion (241,242). Transient expression of the genes c-fos and Egr-1 may code for DNA-binding transcription factors and initiate the transcription of other genes necessary for cell division (243). JE and KC, growth factor-responsive genes with cytokine-like properties that play a role in inflammation, are also expressed during early renal ischemia (244). These genes may code for proteins with chemotactic effects that can attract monocytes and neutrophils into areas of injury (242). Studies demonstrate that c-fos and c-jun are expressed following renal ischemia as a typical immediate early gene response, but they are expressed in cells that do not enter the cell cycle (245,246). The failure of the cells to enter the cell cycle may depend on the co-expression of other genes.

The pathways that lead to the early gene response are interesting. At least two quite different pathways lead to the activation of c-jun (247–249). Growth factors activate c-jun via the mitogen-activated protein kinases (MAPKs), which include extracellular regulated kinases (ERKs) 1 and 2. This pathway is proliferative in nature. In contrast, the stress-activated protein kinase (SAPK) pathway is separate from the MAPK pathway. These kinases include c-Jun *N*-terminal kinase (JNK) 1 and 2. Activation and the effect on cell fate of the SAPK pathway are very different from the MAPK pathway. The SAPK pathway is essentially antiproliferative and can lead to either cell survival or cell death. During renal ischemia, SAPKs are activated, and inhibition of SAPKs after ischemia protects against renal failure (250,251). Thus, it is possible that manipulation of this pathway could lead to therapies that may ameliorate AKI. Also, exploration of the early gene response in renal ischemia using DNA microarrays and other genome-scale technologies should extend our

knowledge of gene function and molecular biology (252).

Microarray analysis of kidney has given clues to the pathogenesis of AKI (252,253). There was an increase in genes involved in cell structure, extracellular matrix, intracellular calcium binding, and cell division/differentiation in kidneys from mice with AKI (254). In another study in mice with AKI, transcription factors, growth factors, signal transduction molecules, and apoptotic factors demonstrated consistent patterns of altered gene expression in the first 24 hours of postischemic reperfusion (255). In rats with AKI, microarray analysis demonstrated that nine genes were upregulated in the early phase (ADAM2, HO-1, UCP-2, and thymosin β4) and established phase (clusterin, vanin1, fibronectin, heat-responsive protein 12, and FK506) (256). Nine genes were downregulated in the early phase (glutamine synthetase, cytochrome p450 IId6, and cyp 2d9) and established phase (cyp 4a14, Xist gene, PPARγ, α-albumin, uromodulin, and ADH B2) Laser capture microdissection of immunofluorescently defined cells (IF-LCM) can isolate pure populations of targeted cells from the kidney for microarray analysis (257). This technique has been used to label and isolate thick ascending limb cells in the kidney for mRNA analysis (257).

Two genes that have been discovered to be activated in the kidney in AKI are kidney injury molecule-1 (KIM-1) in the proximal tubule (258) and neutrophil gelatinase-associated lipocalin (NGAL) in the distal tubules (259–261). KIM-1 is a phosphatidylserine receptor that recognizes and directs apoptotic cells to lysosomes in proximal tubular cells. KIM-1 also mediates phagocytosis of necrotic cells and oxidized lipoproteins by renal proximal tubular cells and increases clearance of the apoptotic debris from the tubular lumen (262). KIM-1 may play an important role in limiting the immune response to injury (262). In early ischemic AKI, KIM-1 expression is antiinflammatory by causing phagocytosis in tubular cells (263). KIM-1-mediated epithelial cell phagocytosis of apoptotic cells protects the kidney after acute injury by downregulating innate immunity and inflammation (263). NGAL is an iron-transporting protein. Purified recombinant NGAL inhibits apoptosis, enhances proliferation, and results in significant functional and pathological protection against AKI in murine models (261). NGAL forms a complex with iron-binding siderophores and stops inappropriately liganded iron from producing damaging oxygen radicals (264).

HYPOXIA-INDUCIBLE FACTOR-1α

Hypoxia-inducible factor-1 (HIF-1)α is an important molecule for the adaptation of cells to low oxygen or hypoxia. Systemic hypoxia, anemia, renal ischemia, or cobalt chloride results in an increase in HIF-1α in renal tubules (265). HIF-1α activation with carbon monoxide protects against ischemic (266) and cisplatin-induced (267) AKI. HIF-1α heterozygous deficient mice have worse AKI compared with control mice (268). Treatment of mice with l-mimosine and dimethyloxalylglycine, agents that activate HIF-1α by inhibiting HIF hydroxylases, protects against ischemic AKI in mice (268). Pharmacologic agents that induce HIF-1α may in the future be a potential therapy for AKI.

CELL CYCLE

Most normal tubular cells are quiescent at the G0 phase of the cell cycle. In AKI, there is cell proliferation in the damaged renal tubules (269). Death or loss of tubular cells may result in the neighboring cells stretching to cover the denuded area. These neighboring cells become dedifferentiated, and activate the cell cycle, driven by cyclins and cyclin-dependent kinases (CDKs) (262). The newly generated cells can develop into polarized, functional tubular cells for kidney repair (262). Cell cycle inhibitors like p21 are also induced during AKI resulting in G1 phase cell cycle arrest (269–272). P53 is another major cell cycle inhibitor that is rapidly induced in AKI is p53 (271,272). Transient cell cycle activation followed by cell cycle arrest may contribute to the development of fibrosis and loss of kidney function after AKI (262). Some tubular cells may become arrested at the G2/M phase, acquire a senescence-like phenotype and produce factors that promote fibrosis. The G1 cell cycle arrest factors, insulin-like growth factor-binding protein-7 (IGFBP7) and tissue inhibitor of metalloproteinase-2 (TIMP-2), in the urine are biomarkers of AKI (see later section on biomarkers of AKI).

The CDK inhibitor p21 was investigated in ischemic AKI and ischemic preconditioning (273). Ischemic AKI and renal histology was worse in the p21 knockout than in wild-type mice. Ischemic preconditioning attenuated I/R injury in wild-type but not p21-knockout mice. Ischemic preconditioning increased renal p21 expression and the number of cells in the G1 phase of the cell cycle before ischemic AKI demonstrating that renal p21 is essential for the beneficial effects of renal ischemic preconditioning. Transient cell cycle arrest induced by ischemic preconditioning by a p21-dependent pathway is important for subsequent tubular cell proliferation after I/R (273).

MITOCHONDRIA

Mitochondrial dynamics are markedly altered in ischemic and nephrotoxic AKI (269). Mitochondrial fragmentation arises before overt renal tubular injury or cell death (274). There is rapid fragmentation of mitochondria by a dynamic process termed fission regulated by proteins such as dynamin-related protein 1 (Drp1) and mitochondrial fission 1 protein (Fis1). Mitofusin 1 (Mfn1) and Mfn2 play a role in mitochondrial fusion. Fragmented mitochondria are a less efficient source of ATP and can undergo the mitochondrial permeability transition which results in influx of water and mitochondrial swelling, cell death through the release of calcium, cytochrome c, proapoptotic proteins, and reactive oxygen species (ROS) (269). Mitophagy results in recycling of damaged mitochondria. AKI is associated with an excess of mitochondrial fission compared with fusion. Pharmacologic inhibition of DRP1 improves mitochondrial morphology and protects against ischemic AKI and improved mitochondrial morphology (275). There is increased mitophagy in AKI to repair or clear fragmented mitochondria. In this regard, the autophagy molecules sestrin-2 and BNIP-3 are upregulated as seen on immunohistochemistry and immunoblot analysis in the ischemic AKI suggesting that autophagy is induced in renal tubules by at least two independent pathways involving p53-sestrin-2 and HIF-1α-BNIP3 (276).

In cisplatin-induced AKI, both oxidative stress and mitochondrial damage are associated with reduced levels of renal sirtuin 3 (SIRT3) (277). Treatment with the AMP-activated protein kinase (AMPK) agonist AICAR or the antioxidant agent acetyl-L-carnitine (ALCAR) restored SIRT3 expression and activity, improved renal function, and decreased tubular injury in wild-type mice but had no effect in Sirt3(−/−) mice (277). Sirt3-deficient mice had worse AKI. In cultured human tubular cells, cisplatin reduced SIRT3, resulting in mitochondrial fragmentation, while restoration of SIRT3 with 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) and ALCAR improved cisplatin-induced mitochondrial dysfunction. This study suggests that SIRT3 improves mitochondrial dynamics in AKI (277).

TUBULAR OBSTRUCTION IN RENAL CELL INJURY

Increased excretion of tubular epithelial casts is a hallmark of recovery from AKI (201). The presence of tubular casts on renal biopsy as well as urinary casts has provided morphologic support for a role of tubular obstruction due to intraluminal cast formation in the pathogenesis of

ischemic AKI (278). Finn and Gottschalk using micropuncture techniques during saline loading demonstrated clear evidence of increased tubular pressures in postischemic compared with normal kidneys (279). Renal vasodilation to restore renal blood flow also demonstrated increased tubular pressures in ischemic AKI in the rat. Tanner and Steinhausen (280) found that perfusing the proximal tubule with artificial tubular fluid at a rate that did not increase tubule pressure in normal animals increased tubule pressures in animals after a renal ischemic insult. Moreover, venting those obstructed tubules led to improved nephron filtration rates. Burke et al. also demonstrated that prevention of ischemic AKI in dogs with mannitol led to a decrease in intratubular pressures, suggesting that the induced-solute diuresis led to relief of cast-mediated tubular obstruction (281).

While it is clear that brush border membranes, necrotic cells, viable cells, and perhaps apoptotic tubular epithelial cells enter tubular fluid after an acute renal ischemic insult, the actual process and predominant location of the cast formation is, however, less clear. In AKI, distal tubules are obstructed by casts formed by tubular debris, cells, and Tamm–Horsfall protein (THP) (278). Since there are arginine–glycine–aspartic acid (RGD) adhesive sequences in human THP, there may be direct integrin-mediated binding of tubular cells to THP. Alternatively, polymerization of THP may result in entrapment of the cells in its gel. Adhesion of LLC-PK(1) cells to THP-coated wells was directly measured, and THP concentrate was dissolved in solutions of high electrolyte concentration that mimic urine from AKI and collecting ducts (282). LLC-PK(1) cells did not directly adhere to THP, a finding against integrin-mediated binding as a mechanism for in vivo tubular cell/THP cast formation. The high electrolyte concentration of AKI solutions was associated with THP gel formation. Thus, with renal ischemia and proximal tubule cell shedding, AKI and collecting duct fluid composition enhance THP gel formation and thus favor tubular cast formation and obstruction.

Integrins also play a role in cast formation. They recognize the most common universal tripeptide sequence, RGD, which is present in a variety of matrix proteins (283). These integrins can mediate cell-to-cell adhesion via an RGD-inhibitable mechanism (284). Experimental results support a role for adhesion molecules in the formation of casts. It has been shown that a translocation of integrins to the apical membrane of tubular epithelial cells may occur with ischemia (284–286). Possible mechanisms for the loss of the polarized distribution of integrins include cytoskeletal disruption, state of phosphorylation, activation of proteases, and

production of NO (287,288). These integrins are known to recognize RGD tripeptide sequences (289,290). Thus, viable intraluminal cells could adhere to other luminal or paraluminal cells. There is experimental evidence for this cell-to-cell adhesion process as a contributor to tubule obstruction in ischemic AKI. Synthetic cyclical RGD peptides were infused before the renal ischemic insult in order to block cell-to-cell adhesion as a component of tubule obstruction (291–295). Using micropuncture techniques the cyclic RGD tripeptides blocked the rise in tubular pressure postischemic insult (289). An in vivo study of RGD peptides in ischemic AKI in rats demonstrated attenuation of renal injury and accelerated recovery of renal function (291). Systemic administration of fluorescent derivatives of two different cyclic RGD peptides, a cyclic Bt-RGD peptide and a linear RhoG-RGD peptide, infused after the release of renal artery clamp ameliorated ischemic AKI in rats (292,294). The staining of these peptides suggests that cyclic RGD peptides inhibited tubular obstruction by predominantly preventing cell-to-cell adhesion rather than cell-to-matrix adhesion (290).

ROLE OF ABNORMAL VASCULAR FUNCTION IN AKI

In organ ischemia, the restoration of perfusion may add to the problem of organ injury. Organ dysfunction attributable to reperfusion has been demonstrated in the heart, lung, brain, intestine, liver, and other organs. The importance of these findings is in their probable contribution to clinical features of myocardial infarction, AKI, and stroke. The implications of reperfusion injury are important in the clinical settings of flow diversion in surgical bypass and for function of transplanted heart, lung, kidney, and other organs.

Injury induced by I/R leads to organ dysfunction, in part by direct injury of parenchymal cells. Vascular dysfunction is an early and prominent aspect of I/R injury, with consequent impairment of blood flow and its regulation. For instance, there may be a progressive loss of regional organ blood flow following I/R. There also may be an exaggerated constriction to neurohumoral agonists, failure to respond to physiologic and pharmacologic vasodilators, and paradoxical vasoconstrictor responses to changes in arterial pressure and blood flow following a period of transient organ ischemia and reperfusion. Evidence suggests that disordered vascular function subsequent to I/R injury may itself have a substantial impact on organ recovery, since normalization of blood flow influences the rate of parenchymal cell restoration.

Table 10–5 Factors That Modify Vascular Tone

Endocrine or Neural
Renal nerves
Catecholamines
Angiotensin II
Natriuretic peptides
Paracrine
Endothelial derived, e.g., nitric oxide, endothelin-1
Angiotensin II
Arachidonic acid metabolites, e.g., thromboxane A_2, prostaglandins, leukotrienes
Purinoreceptors and vasoactive purine agonists, e.g., P1 receptors and adenosine
Dopamine and serotonin

Normal Vascular Tone and Reactivity

Basal vascular tone is essential for perfusion of complex and distinct vascular beds and is dictated in large part by metabolic requirements of individual organs. It is clear that both transmural pressure and shear stress from blood flow contribute to basal arterial vascular tone. The predominant effect of vessel wall pressure is to increase tone; that of flow is to reduce tone. The mechanisms mediating the tonal response to these physical forces are only partially understood. Ca^{2+} entry, at least in part, through unique stretch-operated channels is important in pressure-induced vasoconstriction. VSMC transmembrane Na^+ concentrations are a factor in flow-related vasodilation. In addition, endothelial factors (NO, prostaglandins) are involved in flow-related vasodilation. Aside from its role in mediating shear-induced vasodilation, evidence indicates that endothelial-generated NO independently contributes to normal vascular tone. Other neurohumoral factors that contribute to changes in arterial tone dictated by metabolic demand are adenosine, oxygen, and carbon dioxide (296). Factors that modify vascular tone are listed in Table 10-5.

Vascular Dysfunction due to I/R Injury

The kidney model that exemplifies I/R injury is ischemia-induced AKI. A severe form of this disorder in which the renal artery is clamped for 40 to 70 minutes followed by immediate reflow (297,298) and a less severe form

in which high-dose norepinephrine (NE) is infused into the renal artery for 90 minutes with slow spontaneous return of blood flow (297,299) have been studied extensively in rats. In the clamp model, there is a brief postocclusion hyperemia, then a sustained small reduction in renal blood flow, and an attenuated response to endothelium-dependent dilators (299). In the first few hours after reflow, in the NE model there is a modest reduction in renal blood flow compared with the preischemia level without hyperemia, a decreased response to endothelium-dependent vasodilators, and a small but significant reduction in the constrictor response to the NOS inhibitor L-NAME (296). There is partial endothelial cell detachment without ultrastructural changes in individual endothelial cells at 6 hours in both the renal artery clamp and NE AKI models. By 48 hours of reperfusion, the basal renal blood flow remains 20% reduced in the renal artery clamp model, and there is a reduced vasoreactive response to changes in renal perfusion pressure to constrictor agonists and to endothelium-dependent and endothelium-independent dilators (296). The predominant histologic finding at this time in the small resistance arteries and arterioles is VSMC necrosis, present in 55% to 60% of the vessels (300,301). It is assumed that the lack of response to vasoactive stimuli is due to the diffuse VSMC injury related to both the relative severity of ischemia and the rapidity of reperfusion. In the NE AKI model, at 48 hours, the basal renal blood flow also is approximately 20% less than normal (296,297). However, vascular reactivity is strikingly different from that in the renal artery clamp AKI model. The difference likely is due to less severe ischemia and a slower rate of reperfusion. There is an exaggerated renal vasoconstrictor response to angiotensin II and endothelin-1 (ET-1) both in vivo and in arterioles isolated from these kidneys (296,302). The response to endothelium-dependent vasodilators is reduced, but the constrictor response to L-NAME is actually increased (296). cNOS can be identified as at least as strongly reactive or more reactive than normal, as determined with cNOS monoclonal antibody in the resistance arterial vessels (303). While there is a dilator response to cyclic adenosine monophosphate-dependent PGI_2 in the 48-hour postischemic renal vasculature, there is no increase in renal blood flow to the NO donor sodium nitroprusside. Taken together, these data indicate that at 48 hours after ischemia in NE AKI in the rat kidney, vascular cNOS activity is not diminished but rather is maximal such that it cannot be stimulated further by endothelium-dependent vasodilators. The available NO under basal conditions has fully activated VSMC-soluble cyclic guanosine monophosphate such that there is no additional response to an

exogenous NO donor.

In examining the mechanism for the constrictor hypersensitivity in the 48-hour postischemic vasculature in NE AKI, measurements of VSMC cytosolic Ca^{2+} have been made in the isolated arterioles from these kidneys perfused at physiologic pressures (302). Compared to similar vessels from sham AKI kidneys, there is a significantly higher baseline and an earlier and greater increase in VSMC Ca^{2+} in response to a normal half-maximal constricting concentration (EC_{50}) of angiotensin II, which correlates with the initially lower and more intense reduction in lumen diameter in the postischemic AKI vessels.

Another novel observation regarding VSMC Ca^{2+} in 48-hour postischemic renal arterioles in vitro is a paradoxical change in VSMC cell Ca^{2+} in response to changes in lumen pressure. In normal afferent and efferent arterioles, increasing lumen pressure (stretch) within an autoregulatory range for these vessels results in an increase in VSMC Ca^{2+}. Conversely, decreasing lumen pressure is associated with a decrease in VSMC Ca^{2+}. In the NE AKI vessels, the reverse relationships are observed. There are also corresponding paradoxical changes in lumen diameter, representing, at least, a loss of the myogenic response and, at most, a “reverse” myogenic response. This abnormal VSMC Ca^{2+} and myogenic response to pressure is suggested to be the basis of the markedly abnormal in vivo autoregulatory response between 48 hours and 1 week after AKI induction that is likely the most significant and clinically relevant I/R disorder of vasoreactivity in the kidney.

It was at first thought that Ca^{2+} channel blockers might be exerting their protective effects entirely at the vascular level by promoting the enhancement of renal blood flow. There are unquestioned renal vascular effects of Ca^{2+} channel blockers, with renal blood flow improving more rapidly after ischemia with Ca^{2+} channel blocker treatment (304). Renal blood flow and glomerular filtration will not decrease as severely during radiocontrast administration in dogs when Ca^{2+} channel blockers are coadministered (305). Ischemic AKI is characterized by a loss of autoregulatory ability, an enhanced sensitivity of renal blood flow to renal nerve stimulation, and injury to the endothelial lining of renal vessels (304). Much of this injury may be related to Ca^{2+} overload in VSMCs and/or endothelial cells, since verapamil and diltiazem partially obviate the loss of autoregulatory capacity and hypersensitivity to renal nerve stimulation (304).

Warm and cold ischemia during transplantation surgery may also contribute to vascular injury, and Ca^{2+} channel blockers are protective in experimental models of these clinical entities (306,307). However, other renal vasodilators such as prostacyclin do not restore autoregulatory integrity or reverse the increased sensitivity to renal nerve stimulation (304). Thus, it also seems that a unique effect of Ca^{2+} channel blockers is exerted at the vascular level.

At 1 week after ischemic injury, the endothelium appears normal, smooth muscle necrosis is less evident, but perivascular fibrosis is marked in the mid- to small-sized arterial vessels (297). Functionally, the response to endothelium-dependent dilators is reduced, L-NAME constrictor response is increased, and immunologically detectable NOS is present (303). There is a decreased dilator response to sodium nitroprusside but a measurable, albeit slightly reduced, dilator response to PGI_2 (303). These findings suggest maximal endothelial cNOS activity similar to that at 48 hours. Unlike 48-hour vessels, the vasoconstrictor response to angiotensin II was markedly attenuated both in vivo and in vitro at 1 week (296,308). On the other hand, as previously alluded, a paradoxical vasoconstriction to a reduction in perfusion pressure in the autoregulatory range could be demonstrated in vivo. It is difficult to suggest a single mechanism that explains this series of functional aberrations at 1 week. It is likely that more than one pathophysiologic process is operating to produce these complex responses.

Intravital two-photon microscopy has been used to study the microvascular events within the functioning kidney in vivo (309–312). Intravital two-photon microscopy enables investigators to follow functional and structural alterations with subcellular resolution within the same field of view over a short period of time. Endothelial cell dysfunction within the microvasculature was observed and quantified using the infusion of variously sized, differently colored dextrans or proteins. Movement of these molecules out of the microvasculature and accumulation within the interstitial compartment are readily observed during AKI. The FVB-TIE2/GFP mouse, in which the endothelium is fluorescent, has been used to study morphologic changes in the renal microvascular endothelium during I/R injury in the kidney (313). Alterations in the cytoskeleton of renal microvascular endothelial cells correlated with a permeability defect in the renal microvasculature as identified using fluorescent dextrans and two-photon intravital imaging. This study demonstrates that renal vascular endothelial injury occurs in ischemic AKI and may play an important role in the pathophysiology of

ischemic AKI.

In patients with AKI, it has been demonstrated that diminished NO generation by injured endothelium and loss of macula densa neuronal NOS may impair the vasodilatory ability of the renal vasculature and contribute to the reduction in the GFR (314). Fifty patients who had a cadaveric renal transplant were studied: urinary nitrite and nitrate levels were determined, and intraoperative allograft biopsies were performed. In patients with sustained AKI, urinary nitrite and nitrate excretion was lower than in patients without AKI. In the kidney biopsies, eNOS expression diminished from the peritubular capillaries of 6 of 7 subjects in the sustained AKI group but from only 6 of 16 subjects in the recovery group.

Endothelial Injury

Normal epithelium and endothelium are separated by a small interstitial compartment. The endothelium is coated by a glycocalyx. In I/R injury there is swelling of endothelial cells, disruption of the glycocalyx and endothelial monolayer, and upregulation of adhesion molecules such as ICAMs, VCAMs, and selectins, resulting in increased leukocyte–endothelium interactions (262). There is formation of microthrombi in blood vessels and leukocytes migrate through the endothelial cells into the interstitial compartment (262). There are inflammatory cells and interstitial edema in the interstitial compartment. In ATN, the peritubular capillaries have vacuolar degeneration of the endothelial cell, thickening and multilayer basement membrane formation and attachment and penetration of monocytes (262).

Microparticles are cell membrane-derived particles that can promote coagulation, inflammation, and angiogenesis, and play a role in cell-to-cell communication (315). Microparticles are released by endothelial and circulating cells after sepsis-induced microvascular injury and contribute to endothelial dysfunction, immunosuppression, and multiorgan dysfunction—including sepsis-AKI (315). Glomerular endothelial injury, possibly mediated by a decreased vascular endothelial growth factor (VEGF) level, plays a role in the development and progression of AKI and albuminuria in the LPS-induced sepsis in the mouse (316). In AKI, impaired endothelial proliferation and mesenchymal transition contribute to vascular rarefaction and may contribute to the development of chronic kidney disease (CKD) (317).

The role of caspases and calpain in cisplatin-induced endothelial cell death was investigated (146). Cultured pancreatic microvascular

endothelial (MS1) cells were exposed to 10 and 50 µM cisplatin. Cells treated with 50 µM cisplatin had severe ATP depletion, increased caspase-3-like activity, and displayed extensive PI staining indicative of necrosis at 24 hours. The increase in LDH release and the nuclear PI staining with 50 µM cisplatin at 24 hours was reduced by either the pan-caspase inhibitor, Q-VD-OPH, or the calpain inhibitor, PD-150606. Thus, in cisplatin-treated endothelial cells, caspases, the major mediators of apoptosis, can also cause necrosis. A calpain inhibitor protects against necrosis without affecting caspase-3-like activity suggesting that calpain-mediated necrosis is independent of caspase-3.

The causes of endothelial injury in AKI were investigated. Toll-like receptor 4 (TLR4) regulates early endothelial activation in ischemic AKI (318). There was increased TLR4 expression on endothelial cells of the vasa recta of the inner stripe of the outer medulla of the kidney in ischemic AKI in mice (318). Adhesion molecule (CD54 and CD62E) expression was increased on endothelia of wild-type but not TLR4 knockout mice in vivo. Further, the addition of high-mobility group protein B1, a TLR4 ligand released by injured cells, increased adhesion molecule expression on endothelia isolated from wild-type but not TLR4 knockout mice. TLR4 was localized to proximal tubules in the cortex and outer medulla after 24 hours of reperfusion. Thus, both endothelial and epithelial cells express TLR4, each of which contributes to renal injury by temporally different mechanisms during ischemic AKI (318).

In summary, I/R injury is accompanied by dramatic changes in basal and reactive vascular function of the organ involved. Endothelial injury also occurs in ischemic AKI in mice. There are similarities in altered organ vascular function, particularly in the early reperfusion period of 24 to 48 hours, including changes in permeability, decreased basal organ blood flow, hypersensitivity to vasoconstrictor stimuli, and attenuated response to vasodilators. The reduced responsiveness to endothelium-dependent vasodilators may be due to an actual reduction in eNOS activity or to an actual spontaneous maximal NOS/NO activity that cannot be stimulated further by endothelium-dependent agents.

ROLE OF VASODILATORY SUBSTANCES

Endogenous vasodilators are involved in the hemodynamic changes that both initiate and maintain AKI. In this section, the roles of endogenously generated vasodilators in the pathophysiology of ischemic, septic, and nephrotoxic AKI will be considered, as well as the therapeutic use of

vasorelaxing substances in animal models and in clinical AKI.

Prostaglandins

When renal perfusion pressure is reduced, preglomerular arterial resistance decreases and efferent arteriolar resistance increases to maintain glomerular capillary hydraulic pressure and single-nephron GFR relatively constant. The efferent arteriolar constriction is mediated, in large part, through the local renin–angiotensin system (RAS) (319). Activation of the RAS stimulates synthesis of cyclooxygenase products, including the vasodilator prostaglandins PGI_2 and PGE_2 (320). PGI_2 and PGE_2 oppose the constrictor effects of angiotensin II, thereby attenuating the reduction in renal blood flow as renal perfusion pressure declines. The modulating vasodilator effect of prostaglandins in the setting of reduced renal perfusion appears to be greater in afferent than efferent arterioles. When PGI_2 and PGE_2 were administered exogenously during reduced renal perfusion, filtration fraction increased, with better preservation of the GFR than renal blood flow (321,322), suggesting that vasodilator prostaglandins preferentially caused preglomerular vasorelaxation under these conditions.

Prostaglandin synthesis was found to be increased in animal models of ischemic AKI (321,323), aminoglycoside nephrotoxicity (324), sepsis, and endotoxic shock (325,326). The indication that an increase in prostaglandin activity was renoprotective by maintaining glomerular hemodynamics showed that cyclooxygenase inhibitors in these disorders augmented the reduction in renal blood flow and the GFR (327,328).

Other evidence of protection in AKI was the finding that infusion of biologic prostaglandins and their analogs in ischemic (321,322), mercuric chloride (329), and glycerol-induced AKI (330) results in protection against AKI. The prostaglandin E_1 analog, misoprostol, was found to provide significant protection against ischemia-induced renal dysfunction in rats subjected to renal artery occlusion (331). Misoprostol-treated rats had GFRs almost threefold greater than control animals, although renal blood flow and renal vascular resistance were not significantly different. Misoprostol also protected against renal dysfunction in a model of toxic renal injury produced by mercuric chloride. In an in vitro model employing primary cultures of proximal tubule epithelial cells subjected to hypoxia and reoxygenation, misoprostol, prostaglandin E_2, and prostacyclin limited cell death. This study demonstrated that prostaglandins protect renal tubule epithelial cells from hypoxic injury at

the cellular level independent of hemodynamic factors. Another study demonstrated that inhibitors of cyclooxygenase and lipoxygenase pathways exert a direct protective effect against the hypoxia–-reoxygenation-induced cell injury in renal tubules, a model independent of vascular and inflammatory factors (332).

The PGE_1 study group has performed a pilot study with intravenous PGE_1 administered before radiocontrast media in patients with renal impairment (333). Results from this pilot study suggest that intravenous PGE_1 may be used efficaciously and safely to prevent radiocontrast medium-induced renal dysfunction in patients with preexisting impaired renal function.

Natriuretic Peptides

In 1981, the natriuretic effects of an extract of mammalian atrial myocytes were first reported (334). Subsequently, this substance has been characterized as a polypeptide. The primary stimulus to atrial natriuretic peptide (ANP) synthesis and release is distention of the atria, where storage granules have been identified. Infusion of normal saline into human volunteers increases plasma ANP (335), and plasma ANP levels were elevated in edematous states that involved increased intravascular volume and atrial enlargement such as congestive heart failure.

Natural and synthetic ANPs cause dose-dependent reductions in systemic arterial pressure. The mechanism involves both peripheral vasorelaxation and a reduction in cardiac output (336,337). The magnitude of arterial pressure reduction is dependent on the state of basal vascular tone. ANP has been shown to inhibit both secretion and activity of the renin–angiotensin–aldosterone (338) and adrenergic nervous systems (339), as well as that of vasopressin (340) and ET-1 (341).

ANP has an important effect on the kidney. In vivo infusion of ANPs, both synthetic and naturally occurring from a variety of species, markedly increased the GFR while having a proportionately smaller effect on renal blood flow (342). Studies suggest that ANP-induced renal vasorelaxation was specific for the preglomerular arterioles (343,344). Other studies examining the rat renal microvasculature in vitro indicated that ANP not only directly vasodilated the afferent arteriole but also constricted the efferent arteriole (345,346). The tubular natriuretic effects of ANP involve inhibition of sodium and water transport in the loop of Henle, connecting tubules, and collecting ducts. Among other possible mechanisms, ANP has

been shown to interfere with vasopressin effect and alter adenylate cyclase activity.

Other natriuretic peptides have been discovered. Another class of natriuretic peptides is referred to as brain natriuretic peptide (BNP). It has been isolated from both brain and heart (347,348). BNP, which contains 32 amino acids, has diuretic and natriuretic effects similar to ANP, while the hypotensive effect is not as potent. BNP is now FDA approved for clinical use in congestive heart failure (349).

Numerous animal studies have demonstrated a protective effect of ANPs on ischemic and nephrotoxic in models of AKI (345,350–353). ANP is effective in AKI models even when given after the initiating insult.

On the basis of the encouraging animal experimental results and the unique combination of pharmacologic properties, clinical studies were performed. A multicenter, randomized, double-blind, placebo-controlled clinical trial of ANP in 504 critically ill patients with AKI was performed. The patients received a 24-hour intravenous infusion of either ANP or placebo. The primary end point was dialysis-free survival for 21 days after treatment. The administration of ANP did not improve the overall rate of dialysis-free survival in critically ill patients with ATN. However, ANP decreased the need for dialysis in patients with oliguria (354). In a subsequent study, 222 patients with oliguric AKI were enrolled in a multicenter, randomized, double-blind, placebo-controlled trial. There was no statistically significant beneficial effect of ANP in dialysis-free survival or reduction in dialysis in these subjects with oliguric AKI (355). Mortality rates through day 60 were 60% versus 56% in the ANP and placebo groups, respectively. However, 95% of the ANP-treated patients versus 55% of the placebo-treated patients had systolic blood pressures <90 mm Hg during the study drug infusion ($P < 0.001$). The hypotensive effect of ANP in these recent trials no doubt obscured any intrarenal beneficial effect of the compound.

Calcium Channel Blockers

Calcium channel blockers (CCBs), which inhibit voltage-gated Ca^{2+} entry, have been shown to protect against ischemic and nephrotoxic (cisplatinum, gentamicin) AKI in various animal models (356–362). The protective effect involves less renal vasoconstriction and improved renal blood flow. At a tubular level, there is less AKI and improved mitochondrial function. More recently, it has been demonstrated that the CCB benidipine can ameliorate the ischemic AKI in rats and that the renoprotective effect was

associated with the reduction in apoptosis in tubular epithelial cells (363). Diltiazem also improves renal function in endotoxin-induced AKI in the rat (364).

CCBs have also been examined in clinical studies. Gallopamil resulted in more rapid recovery of renal function in five patients with malaria- or leptospirosis-related AKI (365). Other human experience with CCBs has largely been in the setting of renal transplantation. Verapamil improved early graft function when administered to donors before harvesting the kidneys (54). Diltiazem administered to transplant patients immediately after graft placement resulted in better graft function and a lower incidence of posttransplant AKI (366). More recently, it was demonstrated that isradipine results in a better renal function after kidney transplantation (367). However, the protective effect was independent of delayed graft function or acute rejection.

Fractalkine

Proinflammatory cytokines increase expression of the CX_3C chemokine, fractalkine, on injured endothelial cells. The fractalkine receptor (CX_3CR1) is expressed on natural killer (NK) cells, monocytes, and some $CD8^+$ T cells (368). Fractalkine has a mucinlike stalk that extends the chemokine domain away from the endothelial cell surface, enabling presentation of the CX_3C-chemokine domain to leukocytes. Expression of fractalkine enables bypassing of the first two steps of the adhesion cascade (i.e., rolling and triggering) and mediates cell adhesion between circulating leukocytes and endothelial cells as well as extravasation of these cells. Thus, fractalkine serves the dual function of an adhesion molecule and a chemoattractant (368). Fractalkine is a major chemoattractant for NK cells and monocytes but not neutrophils (369). Fractalkine expression is increased in patients with renal tubulointerstitial inflammation, with the strongest expression localized to vascular sites near to macrophage inflammation (370). Fractalkine is a strong candidate for directing mononuclear cell infiltration induced by vascular injury (370). Fractalkine expression is increased in the endothelium of large blood vessels, capillaries, and glomeruli in ischemic AKI (371). Fractalkine receptor inhibition is protective against ischemic AKI (371). Fractalkine expression is also increased in the blood vessels in mouse kidneys exposed to cisplatin (372). However, fractalkine inhibition did not protect against the functional and histologic abnormalities in cisplatin-induced AKI in mice

(372).

Clinical Relevance of I/R Vascular Injury

The course of human ischemia-induced AKI is highly variable. An important and relevant observation regarding the variable duration and, in particular, the prolonged course in AKI patients was made by Solez et al. (14). In individuals with AKI duration of longer than 3 weeks, a prominent finding in biopsy or autopsy specimens was fresh tubular renal ischemic lesions that could not be related to the remote initial ischemic insult. A possible explanation for the fresh ischemic lesions was altered reactivity of the renal vasculature. Abnormal vascular reactivity in established ischemic AKI animal models includes loss of renal blood flow autoregulation. A number of investigators (300,308,373) have found an attenuated autoregulatory response from 2 to 7 days after AKI induction in the renal artery clamp model in rats.

Against the background of these postischemic vascular perturbations is the observation that a decrease in renal perfusion pressure is not associated with autoregulation of either the GFR or renal blood flow (296,303,304,308,374,375). In fact, rather than renal vasodilation, renal vasoconstriction occurs with a fall in renal perfusion pressure in the postischemic kidney. Thus, a degree of hypotension, which is of no clinical significance in the normal kidney, may cause renal damage in the kidney during the recovery phase of AKI. The same increased sensitivity in the postischemic kidney has also been shown to occur with nephrotoxic agents such as aminoglycosides.

These data have important clinical implications, as a modest arterial pressure reduction during the course of this disease, such as frequently occurs with hemodialysis treatment, can actually result in recurrent ischemic injury and prolongation of AKI (376).

VASOACTIVE RESPONSE TO SEPSIS

Sepsis is the most frequent cause of AKI in ICUs (4,17). AKI occurs in approximately 19% of patients with moderate sepsis, 23% of patients with severe sepsis, and 51% of patients with septic shock (19). The combination of AKI and sepsis is associated with a >80% mortality (4).

Complex vasoactive responses occur in septic AKI. Over the past three decades, sepsis has been studied in various species, including rats, dogs, pigs, primates, and humans. Recently a mouse model of septic AKI has

been developed; this model allows the use of newer molecular techniques, including knockout and transgenic mice, to study the pathogenesis of AKI associated with sepsis.

The initial effects of sepsis in causing AKI primarily involve renal vasoconstriction (377). This renal vasoconstriction can be demonstrated in the absence of sepsis-mediated hypotension (377) as well as in the absence of later events, including apoptosis, leukocyte infiltration, and morphologic evidence of coagulation (e.g., glomerular fibrin) (378–381).

There is evidence that several vasoconstrictor and vasodilator pathways are activated during sepsis in various experimental models. During sepsis, the cytokine-mediated induction of NO results in a hyperdynamic state in which systemic vasodilation is associated with a secondary increase in cardiac output (19). The rise in cardiac output, however, may not be maximal for the degree of afterload reduction because of the myocardial depressant effect of cytokines such as TNF-α. The arterial underfilling associated with systemic arterial vasodilation is known to activate the RAS and the sympathetic nervous system (SNS) (382–385). While these events attenuate or abolish any systemic hypotension, they also lead to renal vasoconstriction. The vasoactive events of sepsis are, however, more complex than those initiated by arterial underfilling. The endotoxin-mediated increase in TNF-α is associated with an increase in iNOS (378,386). There is evidence in the endotoxemic rat that the increased NO that results from the upregulation of iNOS exerts a negative feedback on the eNOS in the kidney (387). Moreover, the secondary messenger of NO, cyclic guanosine 5′-monophosphate (GMP) has been shown to increase in the renal cortex during the initial 16 hours of sepsis but then at 24 hours to be downregulated in spite of continued high plasma levels of NO (388). Both of these events, namely, NO-mediated decreased eNOS and downregulation of cyclic GMP, would impair the normal counterregulatory vasodilator pathways that attenuate the renal vasoconstriction associated with activation of the RAS and SNS. ET-1 has been shown to be increased during endotoxemia in several species (389–393). The capillary leak that leads to interstitial edema and decreased plasma volume during endotoxemia has been reversed with ET receptor blockade in the rat, albeit with a decrease in blood pressure (389).

INFLAMMATION

The inflammatory response may play a major role in the pathogenesis of

ischemic ARF (394,395). Both the innate and adaptive immune response is important in the pathophysiology of ischemic injury (262). The innate occurs early, is non–antigen-specific and is composed of neutrophils, monocytes/macrophages, dendritic cells, NK cells, and natural killer T cells (NKT cells) (262). The adaptive response occurs within hours, lasts a few days, and is activated by specific antigens. The adaptive response includes dendritic cell maturation and antigen presentation, T lymphocyte proliferation and activation, and T to B lymphocyte interactions (262).

The role of neutrophils, lymphocytes, macrophages, and NK cells has been studied in AKI and will be discussed in the next section.

Neutrophils

The role of neutrophils in AKI has been addressed in many studies and remains controversial (396). There is evidence that leukocytes, particularly neutrophils, mediate tubular injury in AKI derived from studies that show an accumulation of neutrophils in ischemic AKI and studies demonstrating a beneficial role of anti-ICAM-1 therapy in AKI (397). Rats depleted of peripheral neutrophils by antineutrophil serum were not protected against ischemic AKI (398). In another study, mice depleted of peripheral neutrophils by antineutrophil serum were protected against ischemic AKI (397).

The adherence of neutrophils to the vascular endothelium is an essential step in the extravasation of these cells into ischemic tissue (399). After adherence and chemotaxis, infiltrating leukocytes release ROS and enzymes that damage the cells (399). Activated neutrophils have been shown to enhance the decrease in the GFR in response to renal ischemia, at least in part due to release of oxygen radicals (400–403). In contrast, infusion of oxygen radical-deficient neutrophils from patients with chronic granulomatous disease did not worsen the course of ischemic injury (402). The mechanism by which adherent leukocytes cause ischemic injury is unclear but likely involves both the release of potent vasoconstrictors including prostaglandins, leukotrienes, and thromboxanes (404) as well as direct endothelial injury via release of endothelin and a decrease in NO (26,405).

Increased systemic levels of the cytokines, TNF-α and IL-1, may upregulate ICAM-1 after ischemia and reperfusion in the kidney (397). The administration of a monoclonal antibody against ICAM-1 protected against ischemic AKI in rats (402,406). ICAM-1-deficient mice are protected against renal ischemia (397). Thus, ICAM-1 is a mediator of

ischemic AKI probably by potentiating neutrophil–endothelial interactions. There is also evidence that upregulation of adhesion molecules may contribute to this impaired medullary blood flow postischemic injury (407–409).

P-selectin is another important molecule involved in adherence of circulating leukocytes to tissue in inflammatory states. Renal ischemia has also been shown to be associated with upregulation of endothelial P-selectin with enhanced adhesion of neutrophils (410). A soluble P-selectin glycoprotein ligand prevents infiltration of leukocytes and protects functionally against ischemic AKI (288).

The role of neutrophils in AKI has been explored in many studies and remains controversial (396,411). There is evidence that neutrophils mediate renal tubular injury in AKI. This evidence is derived from studies that show an accumulation of neutrophils in ischemic AKI and studies demonstrating a beneficial role of anti-ICAM-1 therapy in AKI (397). In another study, mice depleted of peripheral neutrophils by antineutrophil serum were protected against ischemic AKI (397). However, rats depleted of peripheral neutrophils by antineutrophil serum were not protected against ischemic AKI (398).

Mice with ischemic AKI were treated with the pan-caspase inhibitor Quinoline-val-asp(Ome)-CH_2-OPH (OPH-001) (412). OPH-001 induced a marked (100%) reduction in BUN and serum creatinine and a highly significant reduction in the ATN score compared to vehicle-treated mice. OPH-001 significantly reduced the increase in caspase-1 activity and IL-18, and prevented neutrophil infiltration in the kidney during ischemic AKI. To further investigate whether the lack of neutrophil infiltration was contributing to the protection against ischemic AKI, a model of neutrophil depletion was developed. Mice were injected with 0.1 mg of the rat IgG2b monoclonal antibody RB6-8C5 intraperitoneally 24 hours before renal pedicle clamp (413). This resulted in depletion of neutrophils in the peripheral blood and in the kidney during ischemic AKI. Neutrophil-depleted mice had a small (18%) reduction in serum creatinine during ischemic AKI but no reduction in the ATN score despite a lack of neutrophil infiltration in the kidney. Remarkably, caspase-1 activity and IL-18 were still significantly increased in the kidney in neutrophil-depleted mice with AKI. Thus, to investigate the role of IL-18 in the absence of neutrophils, neutrophil-depleted mice with ischemic AKI were treated with IL-18-neutralizing antiserum. IL-18-antiserum-treated neutrophil-depleted mice with ischemic AKI had a significant (75%) reduction in serum creatinine and a significant reduction in the ATN score compared with

vehicle-treated neutrophil-depleted mice. These results suggested a novel neutrophil-independent mechanism of IL-18-mediated ischemic AKI (412).

The IL-23/IL-17 and IL-12/IFN-γ cytokine pathways plays a role in abnormal adaptive immunity. The hypothesis was tested that early production of IL-23 and IL-12 following ischemia reperfusion injury (IRI) activates downstream IL-17 and IFN-γ signaling pathways and promotes kidney inflammation in a mouse model of ischemic AKI (414). Deficiency in IL-23, IL-17A, or IL-17 receptor (IL-17R) and monoclonal antibody neutralization of CXCR2, the p19 subunit of IL-23, or IL-17A attenuated neutrophil infiltration in AKI. IL-17A produced by neutrophils was critical for AKI. IFN-γ administration reversed the protection seen in IL-17A (−/−) mice subjected to IRI, whereas IL-17A failed to reverse protection in IFN-γ (−/−) mice. These results demonstrate that the innate immune component of AKI requires dual activation of the IL-12/IFN-γ and IL-23/IL-17 signaling pathways and that neutrophil production of IL-17A is upstream of IL-12/IFN-γ (414).

Lymphocytes

The role of other leukocytes, for example lymphocytes, has recently been reported. Mice with genetically engineered deficiency of both $CD4^+$ and $CD8^+$ T cells demonstrate a marked improvement in renal function and less neutrophil infiltration in the ischemic kidney compared with control mice. Also mice deficient in CD4 T cells, not CD8 T cells, are significantly protected from AKI (415). Direct evidence for a pathophysiologic role of the CD4 T cell was obtained when reconstitution of CD4-deficient mice with wild-type CD4 T cells restored postischemic injury.

However, there is also a study that CD4 T-cell depletion is not sufficient to protect against ischemic AKI (416). Mice were injected with 10 mg/kg of the rat IgG monoclonal antibody GK1.5 IP or vehicle. Complete $CD4^+$ T-cell depletion with GK1.5 was confirmed by flow cytometry of lymph nodes before induction of AKI and at 24 hours of postischemic reperfusion. Serum creatinine and the ATN score were not different in vehicle-treated and CD4 T–cell-depleted mice with ischemic AKI. These results suggest that $CD4^+$ T cells are not required for the development of ischemic AKI. Therefore, the hypothesis was tested that more than one subset of lymphocyte may need to be depleted for

protection against ischemic AKI. T-cell receptor α chain (TCRα) (−/−) mice lack conventional $\alpha\beta$ T cells and are deficient in both $CD4^+$ and $CD8^+$ T cells. TCRα (−/−) mice were not protected against ischemic AKI.

IL-33 is a recently discovered member of the IL-1 family of cytokines. IL-33 is a nuclear protein that is also released into the extracellular space (417). IL-33 is released as an early response to tissue injury (418). Full-length (active) IL-33 specifically binds the ST2R on CD4 T cells and increases secretion of proinflammatory cytokines (419). Thus, IL-33 is a chemoattractant for CD4 T cells. We have observed increased protein expression of full-length IL-33 in the kidney following induction of AKI with cisplatin (420). Compared with cisplatin-induced AKI in untreated mice, mice treated with a soluble ST2 fusion protein that binds IL-33 had fewer CD4 T cells infiltrate the kidney, lower serum creatinine, and reduced ATN and apoptosis. In contrast, administration of recombinant IL-33 (rIL-33) exacerbated cisplatin-induced AKI, measured by an increase in CD4 T-cell infiltration, serum creatinine, ATN, and apoptosis; this did not occur in CD4-deficient mice, suggesting that CD4 T cells mediate the injurious effect of IL-33. Wild-type mice that received cisplatin and rIL-33 also had higher levels of the proinflammatory chemokine CXCL1, which CD T cells produce, in the kidney compared with CD4-deficient mice. Mice deficient in the CXCL1 receptor also had lower serum creatinine, ATN, and apoptosis than wild-type mice following cisplatin-induced AKI. Taken together, IL-33 promotes AKI through CD4 T cell-mediated production of CXCL1. These data suggest that inhibiting IL-33 or CXCL1 may have therapeutic potential in AKI (420).

Studies in the acute high-dose cisplatin model of AKI (25 mg/kg/d for 3 days) demonstrate that an increase in neutrophils (225) and macrophages (372) occurs late in the course of cisplatin-induced AKI and that neither neutrophil (141) nor macrophage depletion (372) is protective. In an acute model of cisplatin-induced AKI in mice without cancer, data demonstrate that CD4 (−/−) mice are protected against AKI (420). A pathophysiologic role for CD4 T cells in an acute model of cisplatin-induced AKI has been also been demonstrated in two other studies (421,422). It was determined whether CD4 (−/−) mice are protected against AKI in a more clinically relevant 4-week chronic model of cisplatin-induced AKI in mice with lung cancer (423). Kidney function, serum NGAL, ATN, and tubular apoptosis score was the same in wild-type mice and CD4 (−/−) mice with AKI. The lack of protection against AKI in CD4 (−/−) mice was associated with an increase in extracellular signal-regulated kinase (ERK), p38, CXCL1 and TNF-α, mediators of AKI and fibrosis, in both cisplatin-treated CD4 (−/−)

mice and wild-type mice. The lack of protection was independent of the presence of cancer or not. Tumor size was double and cisplatin had an impaired therapeutic effect on the tumors in CD4 (−/−) versus wild-type mice. These data warn against the use of CD4 T-cell inhibition to attenuate cisplatin-induced AKI in patients with cancer.

It was determined whether HSP exerts a renoprotective effects through regulatory T cells (Tregs) (424). T cells from heat-preconditioned mice failed to reconstitute ischemic AKI when adoptively transferred to T–cell--deficient nu/nu mice in contrast to T cells from control mice. Tregs were also increased in heat-preconditioned AKI kidneys. Depleting Tregs before heat preconditioning abolished the renoprotective effect, while adoptive transfer of these cells back into Treg-depleted mice partially restored the beneficial effect of heat preconditioning. The renoprotective effect of HSP70 may be partially mediated by a direct immunomodulatory effect through Tregs (424).

Pharmacologic recruitment of Tregs is a potential therapy for ischemic AKI (425). Pretreatment of mice with the naturally occurring sphingosine *N*,*N*-dimethylsphingosine (DMS) was found to increase both tissue-infiltrating T effectors (Teffs, CD4(+)Foxp3(−)) and Tregs (CD4(+)Foxp3(+)) in the early phase of ischemic AKI. Renoprotection was abolished by administration of the Treg-suppressing agents, anti-CTLA-4 or anti-CD25 monoclonal antibodies, suggesting that Tregs play a key role in DMS-induced renoprotection. Thus, Tregs recruited to the kidney by DMS ameliorate AKI (425).

Endogenous Toll-like receptor 9 (TLR9) regulates AKI by promoting Treg recruitment (426). In cisplatin-induced AKI, Tlr9 (−/−) mice developed worse renal injury and exhibited fewer intrarenal Tregs compared with controls. A series of reconstitution and depletion studies defined a role for TLR9 in maintaining Treg-mediated homeostasis in cisplatin-induced AKI. This study identified a pathway by which TLR9 promotes renal Treg accumulation in AKI (426).

The IL-2/anti-IL-2 complex attenuates renal I/R injury through expansion of Treg cells (427). IL-2C administered before ischemic AKI induced Treg expansion in both spleen and kidney, improved renal function, and attenuated histologic renal injury and apoptosis after IRI. Also, IL-2C administration reduced the expression of inflammatory cytokines and attenuated the infiltration of neutrophils and macrophages in renal tissue. Depletion of Tregs with anti-CD25 antibodies abrogated the beneficial effects of IL-2C. IL-2C-administered ischemic AKI also enhanced Treg expansion in spleen and kidney, increased tubular cell

proliferation, improved renal function, and reduced renal fibrosis (427).

Macrophages

Both monocyte/macrophages and NK cells are well-known sources and targets of injurious cytokines and chemokines (428–431). In a model of macrophage depletion using liposomal clodronate, it was demonstrated that macrophages contribute to tissue damage during acute renal allograft rejection (432) and ischemic AKI (371,433,434). Gene therapy in rats expressing an amino-terminal truncated monocyte chemoattractant protein-1 (MCP-1) reduced macrophage infiltration and ATN (435).

It was determined whether macrophages are a source of IL-18 in ischemic AKI in mice (160). On immunofluorescence staining of the outer stripe of the outer medulla, the number of macrophages double-stained for CD11b and IL-18 was significantly increased in AKI and significantly decreased by macrophage depletion using clodronate. Adoptive transfer of RAW 264.7 cells, a mouse macrophage line that constitutively expresses IL-18 mRNA, reversed the functional protection against AKI in both macrophage-depleted wild-type and caspase-1 (−/−) mice. To test whether IL-18 in macrophages is necessary to cause AKI, macrophages in which IL-18 was inhibited were adoptively transferred. Peritoneal macrophages isolated from wild-type mice, IL-18 binding protein transgenic (IL-18 BP Tg) mice, and IL-18 (−/−) mice were used. IL-18 BP Tg mice overexpress human IL-18 BP and exhibit decreased biologic activity of IL-18. Adoptive transfer of peritoneal macrophages from wild-type as well as IL-18 BP Tg and IL-18 (−/−) mice reversed the functional protection against AKI in LEC-treated mice. In summary, adoptive transfer of peritoneal macrophages in which IL-18 function was inhibited reverses the functional protection in macrophage-depleted mice, suggesting that IL-18 from adoptive transfer of macrophages is not sufficient to cause ischemic AKI.

IL-34 and CSF-1 mediate macrophage survival and proliferation. In ischemic AKI in mice, the time-related magnitude of macrophage-mediated AKI and subsequent CKD were markedly reduced in IL-34-deficient mice (436). IL-34 was generated by tubular epithelial cells and promoted macrophage-mediated tubular epithelial destruction during AKI that worsened subsequent CKD via two mechanisms: enhanced intrarenal macrophage proliferation and elevated bone marrow myeloid cell proliferation. Kidney transplant patients with AKI expressed IL-34, c-FMS, and PTP-ζ in tubular epithelial cells and IL-34 expression increased with worse donor kidney ischemia. The study concluded that IL-34-

dependent, macrophage-mediated, CSF-1 nonredundant mechanisms promote persistent ischemic AKI that worsens subsequent CKD (436).

However, another study demonstrated that CSF-1-mediated expansion and polarization of resident renal macrophages/dendritic cells is an important mechanism promoting renal tubule epithelial regeneration after AKI (437). Macrophage colony-stimulating factor (CSF-1 or M-CSF) is important for kidney repair after ischemic AKI (438). CSF-1 is upregulated in tubule epithelial cells in response to AKI. CSF-1 binds to its receptor, CSF1R, in an autocrine and paracrine manner (438). Proximal tubule production of CSF-1 is important for polarizing and skewing macrophages toward an M2 phenotype, and for recovery from AKI (439).

After kidney I/R injury, monocytes home to the kidney and differentiate into activated macrophages. Proinflammatory macrophages contribute to the initial kidney damage. However, an alternatively activated macrophage reparative phenotype may promote normal renal repair (440). Macrophages isolated from murine kidneys during the tubular repair phase after AKI exhibit an alternative activation gene profile that differs from the canonical alternative activation induced by IL-4-stimulated STAT6 signaling. Tubular cell-mediated macrophage alternative activation is regulated by STAT5 activation. Both in vitro and in ischemic AKI in vivo, tubular cells expressed GM-CSF, a known STAT5 activator, and this pathway was required for in vitro alternative activation of macrophages by tubular cells. These data demonstrate that tubular cells can instruct macrophage activation by secreting GM-CSF, leading to a unique macrophage reparative phenotype that supports tubular proliferation in ischemic AKI (440).

Dendritic Cells

In mice, depletion of kidney and spleen macrophages using liposomal clodronic acid prevented AKI, while adoptive transfer of macrophages restored the AKI response (433). To determine whether macrophages or dendritic cells or both play a role in ischemic AKI, we performed ischemic AKI in CD11b-DTR mice that have a diphtheria toxin (DT)-induced depletion of CD11b cells (macrophages) and CD11c-DTR mice that have a DT-induced depletion of CD11c cells (dendritic cells) (441). While liposomal clodronic acid (that depletes both macrophages and dendritic cells)-treated animals had a significant functional protection from AKI, CD11b-DTR and CD11c-DTR mice were not protected against AKI despite a similar degree of renal macrophage and dendritic cell depletion.

Protection against AKI in LEC-treated compared to CD11b-DTR or CD11c-DTR mice was partially explained by differences in proinflammatory cytokine profiles like CXCL1 and MCP-1. These findings suggested that simple depletion of renal macrophage or dendritic cell subpopulations is not protective against ischemic AKI in mice. Another study also demonstrated that macrophage/monocyte depletion by clodronate, but not DT, improves renal I/R injury in mice (442). In this study, clodronate did not deplete CD206-positive renal macrophages and, unlike DT, left resident CD11c-positive cells unchanged while inducing dramatic apoptosis in hepatic and splenic mononuclear phagocyte populations. Abolition of the protection against AKI by administration of DT to clodronate-treated mice suggested that the protective effect of clodronate resulted from the presence of a cytoprotective intrarenal population of mononuclear phagocytes sensitive to DT-mediated ablation (442).

Mice depleted of dendritic cells before or at the time of cisplatin treatment but not at later stages experienced more severe renal dysfunction, tubular injury, neutrophil infiltration, and greater mortality than mice not depleted of dendritic cells (443). This study demonstrates that resident DCs reduce cisplatin nephrotoxicity and its associated inflammation. The role of endogenous IL-10 and dendritic cell IL-10 in cisplatin-mediated kidney injury was investigated (444). Cisplatin treatment caused an increase in renal IL-10R1 expression and STAT3 phosphorylation. IL-10 knockout mice had worse cisplatin-induced AKI, indicating that endogenous IL-10 ameliorates kidney injury in cisplatin nephrotoxicity. Mixed bone marrow chimeric mice lacking IL-10 in dendritic cells showed moderately greater renal dysfunction than chimeric mice positive for IL-10 in dendritic cells. This study demonstrated that endogenous IL-10 reduces cisplatin-induced AKI and associated inflammation and that IL-10 produced by dendritic cells themselves account for the protective effect of dendritic cells in cisplatin-induced AKI (444).

Delivery of IL-10 via injectable hydrogels improves renal outcomes and reduces systemic inflammation following ischemic AKI in mice (445). Injectable hydrogels can be used to deliver drugs in situ over a sustained period of time. An injectable hydrogel with or without IL-10, or IL-10 was injected under the capsule of the left kidney. At 28 days after ischemic AKI, treatment with IL-10 reduced renal and systemic inflammation, serum IL-6, lung inflammation, urine NGAL, renal histology for fibroblast activity, collagen type III deposition, and fibrosis. Thus injectable

hydrogels are suitable for local drug delivery following renal injury, are biocompatible, and help mitigate local and systemic inflammation (445).

Dendritic cell-mediated NKT cell activation is critical in initiating the immune response following ischemic AKI. Adenosine is an important antiinflammatory molecule. Mice with adenosine 2A receptor-deficient dendritic cells are more susceptible to ischemic AKI (446). Administration of dendritic cells treated ex vivo with an A_2AR agonist protected against ischemic AKI I by suppressing NKT production of IFN-γ and by regulating DC costimulatory molecules that are important for NKT cell activation. The study concluded that ex vivo A_2AR-induced tolerized dendritic cells suppress NKT cell activation in vivo and may be a potential cell-based strategy to attenuate ischemic AKI (446).

NK Cells

NK cells are a type of lymphocyte that mediate innate immunity against pathogens and tumors via their ability to secrete cytokines (447). NK cells are unique in their constitutive expression of receptors for cytokines, for example, IL-18, that are produced by activated macrophages (448). NK cells are activated by IL-18 independently of IL-12 (449). NK cells in mice express mostly the same receptors as humans, including NK 1.1. A model of NK cell activation in injured tissues has been proposed (450). In this model, it is hypothesized that NK cells are recruited to sites of injury from the bloodstream. Once in the tissue, NK cells become activated and release cytokines like IL-18 (450). In support of this hypothesis, it is known that NK cells play a role in numerous disease processes (451).

NK cell depletion in wild-type C57BL/6 mice is protective against ischemic AKI (452). Adoptive transfer of NK cells worsened injury in NK, T, and B cell-null Rag2(−/−) γ(c)(−/−) mice with ischemic AKI. NK cell-mediated kidney injury was perforin (PFN) dependent as PFN(−/−) NK cells had minimal capacity to kill tubular epithelial cells in vitro compared with NK cells from wild-type mice.

Mast Cells

Mast cells are innate immune cells that are involved in immunoglobulin E (IgE)-mediated hypersensitivity, asthma, and host defense against parasites (453). Mast cells are multifunctional pluripotent cells that migrate through vascularized tissues, completing their maturation in the end organs. Mast cells are often located in vascular beds and epithelial surfaces where they

play key roles as sentinels and first responders in host defense. Mast cells contain mediators that are released upon degranulation: cytokines, chemokines, growth factors, leukotrienes, proteases and preformed TNF which can be released immediately after degranulation.

Mast cells mediate AKI through the production of TNF (453). Mast cell-deficient mice with cisplatin-induced AKI had attenuated renal injury, reduced serum levels of TNF, and reduced recruitment of leukocytes to the inflamed kidney. Mast cell-deficient mice also exhibited significantly lower intrarenal expression of leukocyte chemoattractants. Mast cell-deficient mice reconstituted with mast cells from wild-type mice exhibited similar cisplatin-induced renal damage and the same serum levels of TNF as wild-type mice. In contrast, cisplatin-induced AKI was attenuated in mast cell-deficient mice reconstituted with mast cells from TNF-deficient mice The mast cell stabilizer sodium cromoglycate significantly abrogated cisplatin-induced AKI (453).

Renal tubular epithelial cells express increased amounts of the TLRs, TLR2 and TLR4, resulting in increased release of cytokines and chemokines which attract inflammatory cells and modulate the degree of injury (262). The proximal tubular epithelium expresses major histocompatibility complex II resulting in the presentation of antigen to T cells and the expression of costimulatory molecules (262). There is increased expression of B7-1 and B7-2, costimulatory tubule cell molecules on tubular cells that interact with CD28 on T lymphocytes and facilitate cytokine production (262).

The activation of innate immunity involves the release of pathogen-associated molecular patterns (PAMPs) and their binding to pattern recognition receptors (e.g., TLR4). Damage-associated molecular patterns (DAMPs) are molecules that are released dying cells activate cellular receptors resulting in inflammation (454). Proximal tubular cells are sensors of both DAMPs and PAMPs using pattern recognition receptors like TLR4 (455,456). Sepsis induces changes in the expression and distribution of TLR4 in the rat kidney (457). TLRs also play a critical role in ischemic injury, because loss of epithelial TLR4 and MyD88 (458) results in decreased cytokine and chemokine production, decreased inflammation in the kidney, and improved kidney function in ischemic AKI. Thus, TLR-mediated LPS signaling in proximal tubular injury results in the early initiation of damage-associated signaling cascades involving DAMPs and PAMPs. The best characterized DAMP is high-mobility group box 1 (HMGB1) protein which is a ubiquitously expressed nonhistone DNA-binding protein that regulates transcription and is a

proinflammatory mediator (454). HMGB1 is chemotactic for immune cells and inflammatory cells. HMGB1 binds to the receptor for advanced glycation end products (RAGE) and causes NF-κB-induced transcription through interactions with TLRs and RAGE. Other DAMPs include S100 protein, uric acid, galectins, ATP and adenosine, thioredoxin, the intranuclear cytokine, IL-33 and THP (or uromodulin) (454). A neutralizing anti-HMGB1 antibody is functionally and histologically protective against ischemic AKI in mice associated with less tubulointerstitial infiltration by neutrophils (day 1) and macrophages (day 5) and markedly reduced apoptosis of tubular epithelial cells (459). Anti-HMGB1 antibody-treated IRI kidneys had significantly lower levels of IL-6, TNF-α, and monocyte chemoattractant protein 1 (MCP1). Administration of recombinant HMGB1 worsened ischemic AKI. Protection against AKI in TLR4-deficient mice was not affected by administration of either anti-HMGB1 antibody nor recombinant HMGB1 demonstrating that endogenous HMGB1 promotes ischemic AKI possibly through the TLR4 pathway (459). In another study, wild-type mice pretreated with recombinant HMGB1 before ischemia were functionally and histologically protected against ischemic AKI associated with less tubulointerstitial neutrophil and macrophage infiltration, and less tubular epithelial cell apoptosis (460). Gene expression of TLR downstream cytokines and chemokines were also significantly reduced. HMGB1 preconditioning provided additional protection to TLR2 but not TLR4 knockout mice. The protective effect of rHMGB1 preconditioning involved Siglec-G upregulation, a negative regulator of HMGB1-mediated TLR4 pathway activation.

NF-κB

NF-κB is a transcription factor that regulates the expression of many genes involved in immune and inflammatory processes and cell survival (461,462). The effect of direct inhibition of NF-κB transcriptional activity on kidney function, kidney inflammation, tubular apoptosis, and necrosis following the administration of cisplatin was determined (463). Mice were treated with JSH-23 (20 or 40 mg/kg) which directly affects nuclear factor-κB (NF-κB) transcriptional activity. Kidney function, tubular necrosis, but not tubular apoptosis and MPO activity were significantly improved by JSH-23 (40 mg/kg). Sixty-one NF-κB-responsive genes were increased by cisplatin of which 21 genes were decreased by JSH-23. Genes that were decreased by JSH-23 that are known to play a role in cisplatin-induced

AKI were IL-10, IFN-γ, chemokine [C-C motif] ligand 2 (CCL2), and caspase-1. Another gene, caspase recruitment domain family, member 11 (CARD11), not previously known to play a role in AKI, was increased more than 20-fold and completely inhibited by JSH-23. CXCL1 and TNF-α, known mediators of cisplatin-induced AKI, were decreased by JSH-23. RIPK1 and 3, receptor-interacting serine/threonine-protein kinases, that play an important role in necroptosis, were decreased by JSH-23. Thus, NF-κB transcriptional inhibition in cisplatin-induced AKI ameliorates kidney function and ATN without a significant effect on apoptosis and is associated with a decrease in proinflammatory mediators and CARD11.

Tubular epithelial NF-κB activity regulates ischemic AKI (464). There was widespread NF-κB activation in renal tubular epithelia and in interstitial cells that peaked 2 to 3 days after ischemic AKI. Mice expressing the human NF-κB super-repressor IκBαΔN in renal proximal, distal, and collecting duct epithelial cells were protected against AKI tubular apoptosis, and neutrophil and macrophage infiltration. Primary proximal tubular cells isolated from IκBαΔN-expressing mice and exposed to hypoxia-mimetic agent cobalt chloride exhibited less apoptosis and expressed lower levels of chemokines than cells from control mice. The results indicate that in ischemic AKI NF-κB activation in renal tubular epithelia aggravates tubular injury and exacerbates a maladaptive inflammatory response (464).

Adiponectin is a multifunctional cytokine that has a role in regulating inflammation. Adiponectin knockout mice were functionally and histologically protected against ischemic AKI (465). Knockout of adiponectin was found to inhibit the infiltration of neutrophils, macrophages, and T cells into the injured kidneys. This was associated with inhibition of NF-κB activation and reduced expression of the proinflammatory molecules IL-6, TNF-α, MCP-1, and MIP-2 in the kidney. Wild-type mice engrafted with adiponectin-null bone marrow had less AKI than adiponectin-null mice engrafted with wild-type bone marrow. Conversely, adiponectin-null mice engrafted with wild-type bone marrow had similar renal dysfunction and tubular damage compared with wild-type mice engrafted with wild-type bone marrow. These results demonstrate that adiponectin plays a role in the pathogenesis of AKI perhaps via NF-κB activation and that adiponectin may be a potential therapeutic target (465).

Uric Acid

The hypothesis has been presented that uric acid, at levels that do not cause tubular obstruction, may contribute to AKI (466). There are a number of mechanisms by which uric acid may contribute to AKI. Uric acid induces inflammation. Uric acid increases production of the chemotactic factor MCP-1 in VSMCs and C reactive protein synthesis in human vascular endothelial and smooth muscle cells (467). Hyperuricemic rats have a significant increase in macrophage infiltration in their kidneys independent of crystal deposition (468). Renal vasoconstriction also occurs in rats with experimentally induced hyperuricemia. The vasoconstriction is caused by an increase in resistance of the afferent (and, to a lesser extent, efferent) arterioles and a reduction in the single-nephron GFR, which can be attenuated by lowering the uric acid with allopurinol (469). The vasoconstriction is reversed by L-arginine, suggesting that a loss of NO in endothelial cells may be the cause of the vasoconstriction (470). In summary, uric acid may have vasoconstrictive, proinflammatory and pro-oxidative properties that could promote the development of AKI.

Adenosine

Extracellular adenosine is derived mainly *via* phosphohydrolysis of adenosine 5′-monophosphate (AMP) by ecto-5′-nucleotidase (CD73). Extracellular adenosine plays an antiinflammatory role, especially during conditions of limited oxygen availability. The four known adenosine receptor (AR) subtypes (A_1, A_{2a}, A_{2b}, A_3) are expressed in the kidney (471). CD73-dependent adenosine production plays a crucial role in the regulation of the tubuloglomerular feedback (472). It has been demonstrated that protection from ischemic AKI in mice by adenosine A2A agonists or endogenous adenosine requires activation of receptors expressed on bone marrow-derived cells (473). In addition, adenosine A2A agonists mediate protection against ischemic AKI by an action on CD4 T cells (474). Activation of the adenosine A1A receptor plays a protective role in ischemic AKI. Adenosine A1 receptor knockout mice demonstrate increased ischemic AKI (471), and adenosine A1 receptor activation inhibits inflammation, necrosis, and apoptosis in ischemic AKI in mice (475). The adenosine A2B receptor antagonist PSB1115 blocks renal protection induced by ischemic preconditioning, whereas treatment with the selective adenosine A2B receptor agonist BAY 60-6583 dramatically improves renal function and histology following ischemia alone (476). Adenosine A2B receptors were exclusively expressed in the renal vasculature (476). Studies using A2BAR bone marrow chimera

conferred kidney protection selectively to renal A2BARs. These results identify the A2BAR as a novel therapeutic target for providing potent protection from renal ischemia. Pharmacologic or gene-targeted inhibition of CD 73 abolishes renal protection induced by ischemic preconditioning, and treatment of mice with soluble 5′-nucleotidase restores the renal protection induced by ischemic preconditioning (477). In summary, ARs are novel therapeutic targets in ischemic AKI.

The regulatory function of extracellular adenosine on renal perfusion was investigated (478). Equilibrative nucleoside transporters (ENTs) terminate adenosine signaling and it was observed that ENT inhibition in mice elevated renal adenosine levels and protected against ischemic AKI. ENT1 knockout mice were protected against AKI. Crosstalk between renal Ent1 and Adora2b expressed on vascular endothelia effectively prevented the postischemic no-reflow phenomenon seen in AKI. These studies identified ENT1 and ARs as important in reestablishing renal perfusion following ischemic AKI (478). These studies provide novel insight into the preservation of postischemic renal perfusion (479). Endothelial cell adenosine A2B receptors are antagonized by adenosine reuptake into proximal tubule cells by equilibrative nucleotide transporter 1 that can be inhibited by dipyridamole (479). Adenosine A2B receptor agonists and inhibition of ENTs by dipyridamole may offer therapeutic avenues in ischemic AKI (479).

THERAPEUTIC ROLE OF GROWTH FACTORS

The growth factors insulin-like growth factor (IGF-1), epidermal growth factor (EGF), and hepatocyte growth factor (HGF) are known to bind specific receptors in the proximal tubule and regulate metabolic, transport, and proliferative responses in these cells (480). Studies in this area have fallen into two broad categories: (a) those that have examined the renal expression of genes encoding growth factors or transcriptional factors associated with the growth response that is induced after AKI and (b) those that have examined the efficacy of exogenously administered growth factors in accelerating recovery of renal function in experimental models of AKI (481). EGF, HGF, and IGF-1 accelerate the recovery of renal function and regeneration of damaged proximal tubular epithelium and improve mortality in postischemic rat tubular injury (482–484). IGF-1 attenuates delayed graft function in a canine renal autotransplantation model (485). A relationship between expression of antiapoptotic Bcl-2 genes and growth factors in ischemic AKI in the rat has recently been

described (222). It has been demonstrated that antiapoptotic Bcl-2 genes as well as both EGF and IGF-1 are upregulated in the surviving distal tubules and are detected in the surviving proximal tubules, where these growth factors are not usually synthesized (222,486).

The role of epidermal growth factor receptor (EGFR) activation in the recovery from acute ischemic AKI was investigated. Mice with a specific EGFR deletion in the renal proximal tubule—EGFR(ptKO)—were generated (487). Renal function recovery was markedly slowed in EGFR(ptKO) knockout mice. At day 6 after ischemic AKI, there was minimal evidence of injury in the control mice while both EGFR(ptKO) and erlotinib, an EGFR inhibitor, -treated mice still had persistent proximal tubule dilation, epithelial simplification, and cast formation. This study provides both genetic and pharmacologic evidence that proximal tubule EGFR activation plays an important role in the recovery phase after ischemic AKI (487).

A clinical study on IGF-1 in AKI has been performed. The study was designed as a randomized, double-blind, placebo-controlled trial in ICUs in 20 teaching hospitals (488). Seventy-two patients with AKI were randomized to receive recombinant IGF-1 (rhIGF-I) (35 patients) or placebo (37 patients). In this study, rhIGF-I did not accelerate the recovery of renal function in severely ill AKI patients.

HGF is a growth factor that promotes repair and regeneration. Mice with a knockout of the HGF receptor, c-met, had worse cisplatin or ischemic-induced AKI (489). c-met knockout mice had higher serum creatinine, more severe ATN, and increased apoptosis, associated with increased expression of Bax and FasL and decreased phosphorylation/activation of Akt and increased chemokine expression and renal inflammation. Overexpression of HGF in vivo protected against AKI in control mice, but not in Ksp-met(−/−) mice (489).

THERAPEUTIC ROLE OF MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) have a well-known role in regeneration and immunomodulation. A search of clinicaltrials.gov revealed 40 clinical trials of MSCs in patients with Crohn disease, multiple sclerosis, graft versus host disease, ischemic stroke, organ rejection, cartilage repair, lupus nephritis, and heart disease. Administration of MSCs protects against ischemic AKI in rats (490). In this study, the expression of IL-1β, TNF-α, IFN-γ, and iNOS was significantly reduced by intravenous administration of MSCs. In addition, the beneficial effects of MSCs were found to be

mediated by paracrine actions and not by their differentiation into target cells. Human MSCs improve renal function and survival in mice with cisplatin-induced AKI (491). Treatment of mice with autologous and allogeneic MSCs after AKI was safe and reduced renal fibrosis in mice that survived AKI (492). A phase 1 study of MSCs in patients at risk for AKI after cardiac surgery is underway.

The mechanism of the protective effect of MSC therapy in AKI remains unclear. Studies have indicated that MSCs could attenuate inflammation-related organ injury by induction of Tregs. MSCs protected against functional and histologic changes in AKI and downregulated IFN-γ production of T cells in the AKI kidney (493). MSCs increased the percentage of Tregs in the spleen and the ischemic kidney. Antibody-dependent depletion of Tregs blunted the therapeutic effect of MSCs. Coculture of splenocytes with MSCs caused an increase in the percentage of Tregs. Splenectomy abolished attenuation of ischemic injury, and downregulated IFN-γ production and the induction of Tregs by MSCs. Thus, MSCs ameliorate ischemic AKI by inducing Tregs through interactions with splenocytes (493).

Another study investigated the mechanism of the protective effect in AKI of MSCs. Mesenchymal stromal cell-derived extracellular vesicles carrying microRNAs (miRNAs) play a role in protection against AKI (494). Phenotypic changes induced by extracellular vesicles have been implicated in mesenchymal stromal cell-promoted recovery of AKI. miRNAs are potential candidates for cell reprogramming toward a pro-regenerative phenotype. miRNA depletion in mesenchymal stromal cells and extracellular vesicles significantly reduced their intrinsic regenerative potential in AKI, suggesting a critical role of miRNAs in recovery after AKI (494).

COMPLEMENT SYSTEM

There is increased deposition of C3 along the tubular basement membrane in rat and mouse models of ischemic AKI (495). Extrahepatic production of complement proteins, especially by renal tubular epithelial cells, can promote local complement activation and injury. Preclinical studies demonstrate that activation of the complement system is a critical cause of AKI (495). ATN is characterized by activation of the alternative pathway of complement (496). Lack of a functional alternative complement pathway ameliorates ischemic ARF in mice (497). Complement activation within the injured kidney is a proximal trigger of many downstream

inflammatory events in ischemic AKI (495). Complement activation may also account for the systemic inflammatory events that contribute to remote organ injury and patient mortality (495). Complement inhibitory drugs that have entered clinical studies may provide a therapeutic or preventive approach for patients with AKI.

MICRORNAS

miRNAs are short RNAs that regulate gene expression. miRNAs suppress the expression of target genes by binding to the 3′ untranslated regions and inducing repression or degradation of target mRNAs, resulting in reduced protein expression (498). miRNAs play a role in homeostasis as well as causing certain pathological processes. Dicer is a key enzyme in miRNA biogenesis. Mice with a proximal tubular cells knockout of Dicer were markedly protected against ischemic AKI with better renal function, less tubular apoptosis, and better survival compared with wild-type littermates (498). Microarray analysis showed demonstrated that some miRNAs were induced and others were downregulated. Notably, miRNA-132, -362, and -379 showed a continuous change during 12 to 48 hours of reperfusion. miRNA 687 (miR-687) as a key regulator and therapeutic target in ischemic AKI (499). miR-687 is markedly upregulated by HIF-1 in the kidney in ischemic AKI in mice and in hypoxic cultured kidney cells. miR-687 repressed the expression of phosphatase and tensin homolog (PTEN) and facilitated cell cycle progression and apoptosis. Inhibition of miR-687 preserved PTEN expression and attenuated cell cycle activation and renal apoptosis, resulting in functional and histologic protection against ischemic AKI.

An miR integrative network regulating toxicant-induced AKI has been discovered (500). miR-122 was mostly downregulated by cisplatin, whereas miR-34a was upregulated. Foxo3 was identified as a core protein to activate p53. The miR-122 inhibited Foxo3 translation as assessed using an miR mimic, an inhibitor, and a Foxo3 3′-UTR reporter. The role of decreased miR-122 in inducing Foxo3 was confirmed by the ability of the miR-122 mimic or inhibitor to replicate results. Increase in miR-34a also promoted the acetylation of Foxo3 by repressing Sirt1. Cisplatin facilitated the binding of Foxo3 and p53 for activation, which depended not only on decreased miR-122 but also on increased miR-34a. These studies also identified Foxo3 as a bridge molecule to the p53 pathway.

p53 is renoprotective in ischemic AKI by reducing inflammation (501). p53-knockout mice (p53(−/−)) had worse kidney injury, compared with

wild-type mice, and had increased and prolonged infiltration of leukocytes. Acute inhibition of p53 with pifithrin-α in wild-type mice mimicked the observations in p53(−/−) mice. Chimeric mice that lacked p53 in leukocytes sustained injury similar to p53(−/−) mice, suggesting an important role for leukocyte p53 in ischemic AKI. A smaller proportion of macrophages in the kidneys of p53(−/−) and pifithrin-α-treated mice were the antiinflammatory M2 phenotype. Leukocyte p53 is protective by reducing the extent and duration of this inflammation and by promoting the antiinflammatory M2 macrophage phenotype (501).

miRNA-489 induction by HIF-1α protects against ischemic AKI (502). There is miRNA-489 induction in ischemic AKI kidneys. HIF-1α deficiency was associated with decreased miRNA-489 induction in cultured rat proximal tubular cells subjected to hypoxia and kidney tissues of mice after AKI. Inhibition of miRNA-489 increased apoptosis in renal tubular cells after ATP depletion injury in vitro. In mice, inhibition of miRNA-489 enhanced tubular cell death and ischemic AKI. On deep sequencing analysis, there were 417 mRNAs recruited to the RNA-induced silencing complex by miRNA-489, of which 127 contained miRNA-489 targeting sites. Sequence analysis and in vitro studies validated poly(ADP-ribose) polymerase 1 as a miRNA-489 target. These results demonstrate that miRNA-489 is induced via HIF-1α during ischemic AKI (502).

miRNA-24 inhibition reduces ischemic AKI (503). miR-24 was upregulated in the kidney in mice in ischemic AKI and in patients after kidney transplantation. There was specific miR-24 enrichment in renal endothelial and tubular epithelial cells in AKI. Transient overexpression of miR-24 alone in hypoxic cells induced apoptosis whereas silencing of miR-24 ameliorated apoptotic responses. In vitro, adenoviral overexpression of miR-24 targets lacking miR-24 binding sites rescued functional parameters in endothelial and tubular epithelial cells. In vivo, silencing of miR-24 in mice before ischemic AKI resulted in a significant improvement in survival and kidney function, a reduction in apoptosis, improved ATN scores, and less inflammatory cell infiltration in the kidney. Overall, these results indicate miR-24 promotes ischemic AKI by stimulating apoptosis in endothelial and tubular epithelial cells (503).

Hematopoietic miRNA-126 protects against renal I/R injury by promoting vascular integrity (504). Hematopoietic overexpression of miR-126 increased neovascularization of subcutaneously implanted Matrigel plugs in mice. In ischemic AKI in mice, overexpressing miR-126 resulted in a marked decrease in urea levels, fibrotic markers, and injury markers (KIM-1 and NGAL). The protective effect was associated with a higher

density of the peritubular capillary network in the corticomedullary junction and increased numbers of bone marrow-derived endothelial cells. These results demonstrate that overexpression of miR-126 in the hematopoietic compartment is associated with stromal cell-derived factor 1/CXCR4-dependent vasculogenic progenitor cell mobilization and promotion of vascular integrity that supports recovery of the kidney after IRI (504).

ABNORMAL REPAIR OF INJURED PROXIMAL TUBULES AND THE PROGRESSION FROM AKI TO CKD

Epidemiologic and mechanistic studies suggest that the AKI and CKD are not distinct entities but rather are closely interconnected—CKD is a risk factor for AKI, AKI is a risk factor for the development of CKD, and both AKI and CKD are risk factors for cardiovascular disease (505). In a large, community-based cohort of 556,090 adult patients with preexisting normal or near-normal kidney function, an episode of dialysis-requiring ARF was a strong independent risk factor for a long-term risk of progressive CKD and mortality (506). There was a 28-fold increase in the risk of developing Stage 4 or 5 CKD and more than a twofold increased risk of death after dialysis-requiring AKI (506).

The potential mechanisms of the transition from AKI to CKD have been studied in rodent models of AKI. Tubular epithelial cells undergo apoptosis or necrosis or sloughing from the basement membrane in AKI. The surviving cells dedifferentiate, migrate along the basement membrane, proliferate to restore cell number, and then differentiate, in order to restore the functional integrity of the nephron (262). Loss of peritubular microvessels and the chronic activation of macrophages may contribute to interstitial fibrosis (262). The molecular switch that determines whether injured tubular cells undergo repair or a fibrotic response is not known. Epithelial-mesenchymal transition (EMT) may be a major pathways toward fibrosis in other organs (507). However, unequivocal evidence that EMT is a pathway toward renal fibrosis is lacking (508). The injured epithelial cell can produce profibrotic cytokines like TGF-β. In ischemic, toxic, and obstructive models of AKI, a causal association between epithelial cell cycle G2/M arrest and a fibrotic outcome has been demonstrated (509). G2/M-arrested proximal tubular cells activate c-jun NH(2)-terminal kinase (JNK) signaling, which acts to upregulate profibrotic cytokine production and treatment with a JNK inhibitor, or bypassing the G2/M arrest by administration of a p53 inhibitor or the

removal of the contralateral kidney, rescues fibrosis in the unilateral ischemic injured kidney (509). Atrophic tubules that are not recovering have increased signaling of PI3K-Akt-mTOR, ERK-MAPK, JNK-MAPK, and TGF-β pathways, with markedly increased expression of profibrotic peptides PDGF-B, CTGF, and TGF-β (510). In vitro and in vivo studies demonstrate that increased profibrotic TGF-β signaling in tubules recovering from AKI is, in part, attributable to autocrine signaling by lysophosphatidic acid (510). Lysophosphatidic acid signaling acts through separate G protein-coupled receptors triggering $\alpha v\beta 6$ integrin-dependent activation of latent TGF-β as well as transactivation of EGFR and ERK-MAPK (510).

Endothelin plays a role in AKI to CKD transition. Unilateral ischemia caused progressive renal ET-1 protein/mRNA increases with concomitant endothelin A (ETA), but not endothelin B (ETB), mRNA elevations (511). Unilateral ischemia produced progressive renal injury as indicated by severe histologic injury and a 40% loss of renal mass. Pre- or postischemic treatment with the ETA receptor antagonist atrasentan produced protective effects such as decreased tubule/microvascular injury, normalized tissue lactate, and total preservation of renal mass. On the other hand, ETB blockade had no protective effect. These findings provide the first evidence that ET-1 operating through ETA can have a critical role in progression of ischemic AKI to CKD (511).

It is not known whether injury of epithelial cells, endothelial cells, or inflammatory cells plays a role in the AKI to CKD transition. A mouse model of kidney injury using the Six2-Cre-LoxP technology to selectively activate expression of the simian DT receptor in renal epithelia was developed (512). By adjusting the timing and dose of DT, a highly selective model of tubular injury was studied. The DT-induced sublethal tubular epithelial injury was confined to the S1 and S2 segments of the proximal tubule. Acute injury was promptly followed by inflammatory cell infiltration and robust tubular cell proliferation, leading to complete recovery after a single toxin insult. In striking contrast, three insults to renal epithelial cells at 1-week intervals resulted in maladaptive repair with interstitial capillary loss, fibrosis, and glomerulosclerosis, which was highly correlated with the degree of interstitial fibrosis. The study concluded that selective epithelial injury can drive the formation of interstitial fibrosis, capillary rarefaction, and glomerulosclerosis, confirming a direct role for damaged tubule epithelium in the pathogenesis of CKD (512).

The presence of CKD makes AKI worse in mice (513). Sepsis-induced

AKI by cecal ligation was induced in mice with a 5/6 nephrectomy mouse model of progressive CKD. The presence of CKD intensified the severity of kidney and liver injury, cytokine release, and splenic apoptosis. Accumulation of HMGB1, VEGF, TNF-α, IL-6, or IL-10 was increased in CKD or sepsis alone and to a greater extent in CKD-sepsis. Although VEGF neutralization with soluble fms-like tyrosine kinase 1 (sFLT-1) (a soluble VEGF receptor) effectively treated sepsis, it was ineffective against CKD-sepsis. A single dose of HMGB1-neutralizing antiserum did not protect against sepsis alone but attenuated CKD-sepsis. Splenectomy transiently decreased circulating HMGB1 levels, reversing the effectiveness of anti-HMGB1 treatment on CKD-sepsis. The study concluded that CKD increases the severity of sepsis, in part, by reducing the renal clearance of several cytokines. CKD-induced splenic apoptosis and HMGB1 release were found to be mediators for both CKD and sepsis (513).

Blockade of cysteine-rich protein 61 attenuates renal inflammation and fibrosis after ischemic kidney injury (514). In unilateral renal ischemia, increased expression of Cyr61 was detected, predominately in the proximal tubular epithelium. Treatment of mice with an anti-Cyr61 antibody attenuated the upregulation of kidney MCP-1, IL-6, IL-1β, and macrophage inflammatory protein-2, and reduced the infiltration of F4/80-positive macrophages on days 7 and 14 after IRI and reduced expression of collagen, TGF-β, and plasminogen activator inhibitor-I as well as the degree of collagen fibril accumulation, as evaluated by picrosirius red staining, and levels of α-smooth muscle actin proteins by day 14. Treatment of mice with an anti-Cyr61 antibody preserved peritubular microvascular density on day 14. It was concluded that Cyr61 blockade inhibits the triad of inflammation, interstitial fibrosis, and capillary rarefaction after severe ischemic AKI, mechanisms that underlie the AKI to CKD transition (514).

Diagnosis of AKI

THE BIOLOGY BEHIND BIOMARKERS FOR THE DIAGNOSIS AND PROGNOSIS OF AKI

BUN and serum creatinine that are currently used for the diagnosis of AKI are not very sensitive and specific markers of kidney function in AKI as they are influenced by many renal and nonrenal factors independent of

kidney function. Biomarkers that are released into the blood or urine by the injured tubular cells and are analogous to the troponin release by injured myocardial cells after myocardial ischemia or infarction have been studied in detail for the early and more specific diagnosis of AKI. Biomarkers of AKI that have been detected in the urine or serum of patients with AKI and that increase before serum creatinine in AKI include urine IL-18, urine NGAL, urine KIM-1 and urine liver-type fatty acid-binding protein (L-FABP), urinary TIMP2 and IGFBP7 (which is known as NephroCheck—the first FDA-approved biomarker of AKI).

IL-18 is a proinflammatory cytokine that plays a role in both the innate and acquired immune response (515,516). Immunohistochemistry of mouse kidneys demonstrated an increase in IL-18 protein in injured tubular epithelial cells in AKI kidneys compared to normal controls. It was also determined that hypoxic proximal tubules had high levels of IL-18 (154). Urine IL-18 was increased in mice with ischemic AKI compared to sham-operated mice (158).

NGAL is a 21-kDa protein of the lipocalin superfamily. NGAL is a critical component of innate immunity to bacterial infection and is expressed by immune cells, hepatocytes, and renal tubular cells in various disease states (517). NGAL is a small secreted polypeptide that is protease resistant and thus may be easily detected in the urine. NGAL protein increases massively in the renal tubules and in the first urine output after ischemic AKI in rats and mice (518).

KIM-1 is a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain. KIM-1 mRNA and protein are expressed at a low level in normal kidney but are increased dramatically in postischemic kidney (258). Urinary KIM-1 is a noninvasive, rapid, sensitive, and reproducible biomarker for the early detection of both cisplatin-induced AKI and ischemic AKI in rats (519).

Cystatin C is a 13-kDa protein produced by all nucleated cells. It is freely filtered by the glomerulus, completely reabsorbed by the proximal tubules and is not secreted by the renal tubules (520). Thus some of the limitations of serum creatinine, for example, effect of muscle mass, diet, gender, and tubular secretion may not be a problem with cystatin C. Cystatin C is best measured by an immunonephelometric assay. Cystatin C is a better marker of GFR than serum creatinine as demonstrated in the following studies (521,522–524).

L-FABPs are a family of carrier proteins for fatty acids and other lipophilic substances such as eicosanoids and retinoids. FABPs facilitate the transfer of fatty acids between extra- and intracellular membranes.

Urinary L-FABP was increased in mice with ischemic AKI and cisplatin-induced AKI (525). L-FABP was evaluated as a biomarker of renal ischemia in both human kidney transplant patients and a mouse model of AKI (526).

TIMP2 is a human gene. TIMP2 is a member of a gene family that encodes proteins that are natural inhibitors of the MMP. Metalloproteinases are peptidases that play a role in degradation of the extracellular matrix. IGFBP7 regulates the availability of insulin-like growth factors in tissues and modulates IGF binding to its receptors. IGFBP7 stimulates cell adhesion and cancer growth. TIMP2 and IGFBP7 are also markers of cell cycle arrest. Renal tubular cells enter a period of G1 cell cycle arrest after ischemia or sepsis (527). It is proposed TIMP2 and IGFBP7 are expressed in the tubular cells in response to DNA damage and other forms of injury. TIMP2 and IGFBP7 block the effect of the cyclin-dependent protein kinase complexes on cell cycle promotion which results in G1 cell cycle arrest for short periods of time to prevent damaged cells from dividing (528).

BIOMARKERS FOR THE EARLY DIAGNOSIS OF AKI

See Table 10-6.

Urine IL-18

Studies in humans demonstrated that urine IL-18 is an early predictive biomarker of AKI (529). The TRIBE-AKI (Translational Research Investigating Biomarkers in Early Acute Kidney Injury) Clinical Consortium was established to hasten the development of biomarkers. In the TRIBE-AKI study, urine IL-18 and NGAL were studied as early biomarkers of AKI in a prospective multicenter observational cohort study of 1,219 patients receiving cardiac surgery (530). It was demonstrated that urine IL-18, urine NGAL, and plasma NGAL associate with subsequent AKI and poor outcomes. Urine IL-18 and urine and plasma NGAL levels peaked within 6 hours after surgery. After multivariable adjustment, the highest quintiles of urine IL-18 and plasma NGAL associated with 6.8-fold and fivefold higher odds of AKI, respectively, compared with the lowest quintiles. Elevated urine IL-18 and urine and plasma NGAL levels associated with longer length of hospital stay, longer ICU stay, and higher risk for dialysis or death. Urine IL-18 and plasma NGAL significantly improved the area under the receiver operating characteristic (ROC) curve

to 0.76 and 0.75, respectively.

Urine NGAL

The usefulness of NGAL as an early biomarker of AKI was reviewed in a meta-analysis. Fifty-eight manuscripts reporting on NGAL as a biomarker of AKI in more than 16,500 patients were analyzed (531). Following cardiac surgery, NGAL measurement in over 7,000 patients was predictive of AKI and its severity, with an overall area under the ROC curve of 0.82 to 0.83. Similar results were obtained in over 8,500 critically ill patients. In over 1,000 patients undergoing kidney transplantation, NGAL measurements predicted delayed graft function with an overall area under the curve (AUC) of 0.87. In all three settings, NGAL significantly improved the prediction of AKI risk over the clinical model alone.

Table 10–6 Biomarkers for the Early Diagnosis AKI

Urine
IL-18
NGAL
KIM-1
Cystatin C
L-FABP
TIMP2/IGFBP7
Brush border enzymes: GGT, Alk Phos, NAG, GST
Albumin:creatinine ratio
Serum
NGAL
Cystatin C

IL-18, interleukin-18; NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1; L-FABP, liver fatty acid-binding molecule-1; TIMP2, tissue inhibitor of metalloproteinases-2; IGFBP7, insulin-like growth factor-binding protein 7; GGT, gamma-glutamyl transferase; Alk Phos, alkaline phosphatase; NAG, *N*-acetyl-*β*-D-glucosaminidase; GST, glutathione *S*-transferase.

KIM-1

In a meta-analysis, urinary KIM-1 was analyzed in 2,979 patients from 11 studies (532). The sensitivity of urinary KIM-1 for the diagnosis of AKI

was 74.0%, specificity was 86.0%, and the area under the ROC curve was 0.86. Population settings and detection time were the key factors affecting the efficiency of KIM-1 for AKI diagnosis.

Cystatin C

In patients with AKI, serum cystatin C rises prior to serum creatinine (533). In the TRIBE-AKI study of 1,203 adults and 299 children undergoing cardiac surgery, it was found that urinary cystatin C was not significantly associated with the development of AKI after cardiac surgery (534).

Urine L-FABP

In the TRIBE-AKI study, urine L-FABP peaked within 6 hours after surgery in both adults and children (535).

BIOMARKERS FOR RISK STRATIFICATION OF PATIENTS WITH EXISTING AKI

The ability to predict whether AKI will progress may improve management, guide patient and family counseling, and identify patients for enrollment into therapeutic trials.

Biomarkers measured on the day of AKI diagnosis improve risk stratification and identify patients at higher risk for progression of AKI and worse patient outcomes (536). AKI biomarkers (IL-18, urine and plasma NGAL, urinary albumin to creatinine ratio) measured at the time of first clinical diagnosis of early AKI after cardiac surgery can determine the severity of the AKI. Using multivariable logistic regression, and after adjustment for clinical predictors, the highest quintiles of the three biomarkers remained associated with AKI progression compared with biomarker values in the lowest two quintiles.

IGFBP7 and TIMP2 were shown to be biomarkers of risk stratification in AKI in three studies: Sapphire, Topaz and Opal studies. Biomarkers were measured in critically ill ICU patients with sepsis or one or more risk factors for AKI, for example, hypotension, sepsis, major trauma. The cell cycle arrest proteins, urinary IGFBP7 and TIMP-2, both inducers of G1 cell cycle arrest, a key mechanism implicated in AKI (537), were able to predict RIFLE I and F within 12 to 36 hours. IGFBP7 and TIMP-2 demonstrated an AUC of 0.80 (0.76 and 0.79 alone) for the primary end

point of moderate to severe AKI (KDIGO Stage 2–3) within 12 hours of sample collection (537). In the Topaz study, a predefined cutoff value of IGFBP7 and TIMP-2 was prospectively validated (538). Critically ill patients with urinary [TIMP-2]×[IGFBP7] greater than 0.3 had seven times the risk for AKI compared with critically ill patients with a test result below 0.3.

Preoperative BNP levels predict postoperative AKI among patients undergoing cardiac surgery (539).

BIOMARKERS OF AKI AND LONG-TERM OUTCOMES

In the TRIBE-AKI study, NGAL, IL-18, KIM-1, L-FABP, and albumin were measured on postoperative days 1 to 3 (540). During a median follow-up of 3.0 years, the highest tertiles of peak urinary NGAL, IL-18, KIM-1, liver fatty acid-binding protein, and albumin were independently associated with a 2.0- to 3.2-fold increased risk for mortality compared with the lowest tertiles. In patients without clinical AKI, the highest tertiles of peak IL-18 and KIM-1 were independently associated with long-term mortality.

TIMP2 and IGFBP7 have been validated for long-term outcomes in AKI. [TIMP-2]×[IGFBP7] levels are associated with adverse long-term outcomes in patients with AKI (541). In a univariate analysis, [TIMP-2]×[IGFBP7] >2.0 was associated with increased risk of a composite end point of all-cause mortality or the need for RRT. In a multivariate analysis adjusted for the clinical model, [TIMP-2]×[IGFBP7] levels >0.3 were associated with death or RRT in subjects who developed AKI. Thus, [TIMP-2]×[IGFBP7] measured early in the setting of critical illness may identify patients with AKI at increased risk of mortality or receipt of RRT over the next 9 months. The measurement of [TIMP-2]×[IGFBP7] has been marketed as NephroCheck and has recently been FDA-approved for the detection of AKI in ICU patients.

BIOMARKERS OF SUBCLINICAL AKI

“Subclinical” AKI is AKI in the absence of an increase in serum creatinine (542). There is evidence that patients with subclinical AKI have worse clinical outcomes (542).

Some biomarkers of AKI are increased in prerenal tubular injury in which there is a transient increase in serum creatinine (543).

In 287 pediatric patients without preoperative AKI or end-stage renal

disease (ESRD) who were undergoing cardiac surgery, compared with the serum creatinine-based definition, the cystatin C-based definition is more strongly associated with urine IL-18 and kidney injury molecule 1 (544).

FIBROBLAST GROWTH FACTOR 23

Fibroblast growth factor 23 (FGF23) plays a major role in phosphate and vitamin D metabolism. The main function of FGF23 is the regulation of phosphate concentration in plasma. FGF23 is secreted by osteocytes in response to elevated calcitriol. FGF23 acts on the kidneys, where it decreases the expression of NPT2, a sodium phosphate cotransporter in the proximal tubule resulting in decreased reabsorption of phosphate. FGF23 also suppress 1-alpha-hydroxylase, reducing its ability to activate vitamin D and thus also impairs calcium absorption.

Circulating FGF23 levels rise rapidly during AKI in rodents and humans (545). In mice, this increase is independent of established modulators of FGF23 secretion (545). In patients undergoing elective cardiac surgery, a simple preoperative FGF23 measurement is a powerful indicator of surgical mortality, postoperative complications, and long-term outcome (546). In an analysis adjusted for age, preoperative eGFR, and cardiopulmonary bypass time, higher cFGF23 levels at the end of cardiopulmonary bypass were significantly associated with greater risk of severe AKI and the need for RRT or death (547). Thus, cFGF23 levels rise early in AKI postcardiac surgery and are independently associated with adverse postoperative outcomes (547). Abnormalities in FGF23 regulation start early in the course of AKI, are in part independent of the increase in serum parathyroid hormone (PTH), and involve the activation of FGFR1 (548). It is possible that FGFR1 in the osteocyte is activated by locally produced canonical FGFs, which are increased in AKI (548). It is unknown whether cFGF23 is simply a marker of severity of illness or whether FGF23 directly contributes to organ injury and adverse outcomes.

Klotho is a single-pass transmembrane protein which functions as a coreceptor for FGF23. Ischemic AKI in rodents reduced Klotho in the kidneys, urine, and blood, all of which were restored upon recovery (549). Patients with AKI were found to have drastic reductions in urinary Klotho. To examine whether Klotho has a pathogenic role, we induced IRI in mice with different endogenous Klotho levels ranging from heterozygous Klotho haploinsufficient, to wild-type, to transgenic mice overexpressing Klotho. Klotho levels in AKI were lower in haploinsufficient and higher in transgenic compared with wild-type mice. Mice deficient in Klotho had

more extensive functional and histologic ischemic AKI, whereas mice overexpressing Klotho have more AKI, indicating that Klotho is renoprotective. Rats with AKI given recombinant Klotho had less AKI. It was concluded that endogenous Klotho is both an early biomarker for AKI and a renoprotective factor in AKI (549).

Cisplatin-induced AKI was exaggerated in Klotho haplosufficient mice and ameliorated in transgenic Klotho-overexpressing mice (550). NGAL and active caspase-3 protein, and number of apoptotic cells in the kidney were higher in Klotho haplosufficient mice and lower in Klotho transgenic mice. Klotho suppressed basolateral uptake of cisplatin by rat kidney cells in culture. Thus, Klotho protects the kidney against cisplatin nephrotoxicity by reduction in the basolateral uptake of cisplatin by OCT2, and a direct antiapoptotic effect independent of cisplatin uptake (550).

In rat kidney and in a rat renal tubular epithelial cell line, transgenic overexpression of Klotho or addition of exogenous recombinant Klotho increased kidney erythropoietin receptor (EpoR) protein and transcript (551). Knockdown of endogenous EpoR rendered NRK cells more prone to injury, and overexpression of EpoR made cells more resistant to peroxide-induced cytotoxicity, indicating that EpoR mitigates oxidative damage. Knockdown of EpoR by siRNA blunted the protective effect of Klotho against peroxide-induced cytotoxicity. This study demonstrates that in the kidney, the EpoR and its activity are downstream effectors of Klotho enabling it to function as a cytoprotective protein against oxidative injury (551).

Table 10–7 Conditions Causing Prerenal Azotemia

Hypovolemia
Hemorrhage
Gastrointestinal losses
Third space
Burns
Peritonitis
Muscle trauma
Renal fluid losses
Overdiuresis
Impaired Cardiac Function
Congestive heart failure
Cardiogenic shock
Acute myocardial infarction

Pericardial tamponade
Massive pulmonary embolism
Systemic Vasodilatation
Gram-negative bacteremia
Antihypertensive medications
Anaphylaxis
Cirrhosis
Increased Renal Vascular Resistance
Anesthesia
Surgery
Hepatorenal syndrome
Prostaglandin inhibitors
NSAIDs
Renal vasoconstricting drugs
Cyclosporin

NSAID, nonsteroidal antiinflammatory drug.

*α*Klotho mitigates progression of AKI to CKD through activation of autophagy (552). Heterozygous *α*Klotho-hypomorphic mice (*α*Klotho haploinsufficiency) progressed to CKD much faster than wild-type mice. *α*Klotho-overexpressing mice had better preserved renal function after AKI. A high phosphate diet exacerbated *α*Klotho deficiency after AKI, dramatically increased renal fibrosis, and accelerated CKD progression. Recombinant *α*Klotho administration after AKI accelerated renal recovery and reduced renal fibrosis. *α*Klotho deficiency and overexpression was associated with lower and higher autophagic flux in the kidney, respectively. The study proposes that *α*Klotho upregulates autophagy, attenuates ischemic injury, mitigates renal fibrosis, and retards AKI progression to CKD (552).

PRERENAL AZOTEMIA

The conditions causing prerenal azotemia are listed in Table 10-7. There are four clinical criteria required for a diagnosis of prerenal azotemia: (a) an acute rise in BUN and/or serum creatinine, (b) renal hypoperfusion, (c) a bland urine sediment (absence of cells and cellular casts), and (d) the return of renal function to normal within 24 to 48 hours of correction of the hypoperfused state.

The azotemic state can be corrected if the renal hypoperfusion causing the renal ischemia is reversed. Such an improvement in renal function may involve increasing extracellular fluid (ECF) volume, enhancing cardiac output, or correcting the cause of systemic arterial vasodilation, such as bacteremia or excessive use of antihypertensive drugs. Correction or improvement of an insult, such as anesthesia, surgical trauma, liver disease, or bilateral renal vascular occlusion, may also reverse a state of prerenal azotemia. A careful search by history and physical examination for causes of prerenal azotemia, therefore, must constitute the initial undertaking in the evaluation of patients with the potential diagnosis of AKI.

A recent study demonstrated that some biomarkers of AKI are increased in prerenal azotemia (543). Urinary biomarkers of injury (cystatin C, NGAL, γ-glutamyl transpeptidase, IL-18, and KIM-1) were measured in patients with no AKI, AKI with recovery by 24 hours, recovery by 48 hours, or the composite of AKI greater than 48 hours or dialysis. Prerenal AKI was identified in 61 patients as recovery within 48 hours and a fractional sodium excretion <1%. Biomarker concentrations significantly and progressively increased with the duration of AKI. The median concentrations of KIM-1, cystatin C, and IL-18 were significantly greater in prerenal AKI compared with no AKI. This study suggests that prerenal AKI represents a milder form of injury (543).

POSTRENAL AZOTEMIA

Obstruction of urine flow in both ureters, the bladder, or urethra may cause postrenal AKI. The common causes of postrenal AKI are listed in Table 10-8. The common denominator of acute azotemia in this setting is obstruction to the flow of urine. The patient most at risk of acute postrenal azotemia is the elderly man in whom prostatic hypertrophy or prostatic cancer may lead to complete or partial obstruction to urine flow. In addition to anatomic causes, functional disturbances of bladder emptying also must be considered. Autonomic insufficiency, spinal cord lesions, and anticholinergic agents may cause functional bladder neck obstruction and thus postrenal azotemia. Young boys with congenital urethral valves may also have acute obstruction. In women, complete urinary tract obstruction is relatively uncommon in the absence of pelvic surgery, pelvic malignancy, or previous pelvic irradiation. A pelvic examination is mandatory in the evaluation of postrenal azotemia because patients with cervical or endometrial carcinoma or endometriosis may present with

azotemia secondary to bilateral ureteral obstruction. A history of analgesic nephropathy, sickle cell anemia, diabetes mellitus, or acute pyelonephritis may suggest obstruction secondary to papillary necrosis.

Table 10–8 Conditions Causing Postrenal Azotemia

Urethral Obstruction
Valves
Stricture
Bladder Neck Obstruction
Prostatic hypertrophy
Bladder carcinoma
Bladder infection
Functional
Autonomic neuropathy
Alpha adrenergic blockers
Obstruction of Ureters, Bilateral Unilateral Obstruction in Solitary Kidney
Intraureteral
Sulfonamide, uric acid, acyclovir, antiretroviral agent crystals
Blood clots
Stones
Necrotizing papillitis
Extraureteral
Tumor of cervix, prostate, bladder
Endometriosis
Periureteral fibrosis
Accidental ureteral ligation
Pelvic abscess or hematoma

In the absence of a single kidney or previously impaired renal function, postrenal azotemia occurs only with bilateral obstruction of the urinary tract at these sites. Renal ultrasonography will detect pelvicalyceal dilatation secondary to obstruction in >90% of patients. Staghorn calculi and small shrunken kidneys, however, decrease this sensitivity, and extrarenal pelvices may produce a false-positive diagnosis. Pelvicalyceal dilatation may not occur in some cases of retroperitoneal fibrosis. With the recognition and increased evidence of radiocontrast-induced AKI, it is

most appropriate to use ultrasonography to exclude urinary tract obstruction. In some cases, retrograde pyelography may be necessary to exclude urinary tract obstruction definitively. The rapidity of the recovery of renal function depends on the duration and completeness of the obstruction.

INTRARENAL OR INTRINSIC AKI

After prerenal and postrenal azotemia have been excluded, the diagnosis of intrarenal or intrinsic AKI can be entertained (Table 10-2). Clinically it can be diagnosed by one of the following:

1. An increase in BUN only (prerenal AKI).
2. An increase in BUN and serum creatinine. Normal BUN is 8 to 18 mg/dL, and normal serum creatinine is 0.6 to 1.2 mg/dL. Serum creatinine should be interpreted in relationship to the muscle mass of the patient.
3. An increase in serum creatinine and decrease in urine output. See RIFLE and AKIN criteria for AKI (Fig. 10-1 and Table 10-1).
4. Oliguria. Oliguria is defined as a urine output <400 mL/day—the minimum amount of urine that a person in a normal metabolic state needs to excrete to get rid of his or her daily solute production. However, in as many as half the cases of AKI, the daily urine volume may exceed this amount and actually be as high as 1.5 to 2.0 L/day (553). This form of AKI has been termed nonoliguric AKI. It is frequently associated with nephrotoxin-induced disease and tends to carry a lower morbidity and mortality than oliguric failure. In nonoliguric renal failure, urinary sodium concentration, fractional excretion of sodium, and the urine to plasma creatinine ratio are lower at the time of diagnosis than in oliguric AKI (554). The exact mechanism for the higher urine flow in this variety of AKI is not known. However, the finding of a higher creatinine clearance in nonoliguric patients suggests that the GFR may be better preserved. Despite liberal daily urine volumes, progressive azotemia with nonoliguric AKI may occur in the following manner. Since the abolition of the renal concentration capacity is a characteristic of AKI, approximately 300 mOsm of solute can be excreted in each liter of isotonic urine. The catabolic rate of patients with AKI is often markedly increased. In these individuals, there may be an exogenous plus endogenous solute load as great as 900 mOsm/day. The daily

excretion of 2 L of isotonic urine thus will eliminate only 600 mOsm of the 900-mOsm solute load. Therefore, despite a daily urine output of 2 L, progressive azotemia will result because of the 300-mOsm daily positive solute balance. Such is the sequence of events that occurs in nonoliguric renal failure.

5. Anuria. Anuria has been defined in the past as a 24-hour urine volume <75 mL and has been suggested to be more compatible with urinary tract obstruction or renal vascular occlusion than with AKI. Such a definition of anuria, however, is probably not appropriate. During the first few days of oliguric AKI, urine volumes may frequently be <75 mL/day when assessed by bladder catheterization. It has been documented that such severe oliguria can occur with AKI in the absence of renal vascular or urinary tract obstruction (555). Anuria, therefore, is best defined as the excretion of no urine as documented by bladder catheterization. Anuria by this definition may suggest bilateral renal artery occlusion and thus the need for emergency renal arteriography, particularly in the appropriate clinical setting, such as atrial fibrillation with arterial emboli, abdominal trauma, or a dissecting aortic aneurysm. Because of the slower progression of irreversible functional loss with urinary tract obstruction, some minimal delay (a few days) in establishing this diagnosis may be acceptable, depending on the clinical status of the patient.

In patients who are not acutely ill, an increased BUN and serum creatinine may be caused by acute or chronic renal failure. Features suggesting chronic renal failure are:

1. Symptoms for longer than 3 months, for example, malaise and nocturia.
2. An increased BUN or serum creatinine documented months earlier.
3. A normocytic normochromic anemia. However, patients with AKI and a microangiopathic hemolytic anemia, for example, hemolytic uremic syndrome (HUS), may have a normocytic anemia.
4. Small kidneys (<10 cm) on renal ultrasound. However, some patients with chronic renal failure, for example, diabetic nephropathy, amyloidosis, autosomal dominant polycystic kidney disease, rapidly progressive glomerulonephritis, or malignant hypertension, may have normal-sized or enlarged kidneys.

EVALUATION OF THE PATIENT WITH AKI

A comprehensive history, thorough physical examination, and urinalysis (sediment and chemistry) will suggest the diagnosis in the majority of patients.

History and Physical Examination

Careful tabulation and recording of data are the first steps in diagnosis and treatment. Vital signs, daily weights, records of intake and output, past and current laboratory data, and the fluid and medication list should be recorded on a flow sheet and included in the patient's chart. When the patient has been hospitalized for weeks or months with a complicated course before developing AKI, a carefully prepared flow sheet may often be the only way to detect causes of AKI, such as administration of nonsteroidal antiinflammatory drugs (NSAIDs) or prophylactic antibiotics.

It is helpful to determine whether the AKI developed outside the hospital, inside the hospital, or in the ICU. The causes and management of AKI may differ in these circumstances. Common causes of AKI developing outside the hospital are acute systemic illness, for example, viral influenza; gastroenteritis that may lead to AKI through a variety of mechanisms, for example, volume depletion; and rhabdomyolysis with myoglobinuria. Trauma as the cause of acute azotemia is usually apparent at the time of admission to the hospital, but the unconscious or comatose patient may harbor internal injuries, extensive muscle damage, or acute urinary retention that is not discovered on the initial examination. Male patients with acute azotemia should be screened carefully for symptoms of prostatism. Aspirin, NSAIDs, antibiotics, and diuretics are the main causes of AIN. They are often prescribed outside the hospital. Accidental or intentional intoxication with heavy metal compounds, solvents, ethylene glycol, salicylates, or sedatives, especially in a patient presenting with disordered mentation, may explain an otherwise unexpected episode of AKI.

When azotemia develops in the hospital setting, but not in the ICU, the list of possible causes narrows. Most of the patients have either ATN (38%) or prerenal azotemia (28%). Predisposing factors to AKI in this setting include fluid and electrolyte depletion, for example, excessive diuresis, nasogastric suction, surgical drains, and diarrhea in a patient who is too ill to control his or her own solute and water intake. Both surgery and anesthesia cause a vasoconstriction of the renal arteries and release of antidiuretic hormone; both of these effects may persist for 12 to 24 hours into the postoperative period. Nephrotoxic drugs and diagnostic agents, for

example, radiocontrast media, represent a major and serious cause of acute azotemia.

The majority of patients who develop AKI in the ICU have ATN (76%), and most of the rest have prerenal azotemia (18%). The mortality rate of these patients is >70%, which is much higher than the mortality of AKI developing in other areas of the hospital. Patients with respiratory failure secondary to acute respiratory distress syndrome (ARDS) requiring mechanical ventilation that subsequently develop AKI in the ICU requiring dialysis have a very high mortality >90% (556,557). In the ICU, AKI is often part of the multiorgan dysfunction syndrome.

Urinalysis

Assessment of the urinary sediment also is crucial in the diagnosis of AKI. An active sediment with renal tubular epithelial cells, cellular debris, and "muddy-brown" broad tubular cell casts supports the diagnosis of ATN. Large amounts of urinary protein (>3.0 g/day) and numerous red blood cell (RBC) casts are indicative of AKI secondary to acute glomerulonephritis or vasculitis. The absence of cellular elements and protein in the urine is most compatible with prerenal and postrenal azotemia. An abundance of crystals in the urine, such as uric acid or oxalate crystals, secondary to ethylene glycol or methoxyflurane toxicity or crystalluria in an acquired immunodeficiency syndrome (AIDS) patient being treated with acyclovir or indinavir also may provide a clue to the specific cause of the AKI.

Considerable information may be obtained from the assessment of the urinary composition (554,558). A study by Miller et al. (554) evaluated the differences in urinary composition between prerenal azotemia and both oliguric and nonoliguric AKI. Those differences are summarized in Table 10-9. Since tubular function is preserved in prerenal azotemia and the tubules are able to reabsorb sodium, the urinary sodium concentration decreases in response to the renal ischemia. In prerenal azotemia, the renal concentrating mechanism also is activated so that the urine osmolality exceeds plasma osmolality. With the intact tubular function in prerenal azotemia and tubular fluid resorption, the urine to plasma (U/P) creatinine ratio >40:1 with prerenal azotemia. This ratio is generally <20:1 in intrinsic or parenchymal AKI. If mannitol or a diuretic has been administered within a few hours of obtaining the urine for examination, the interpretation of the urinary composition is difficult because with prerenal azotemia the administration of either of these substances may raise the

urinary sodium concentration and impair renal concentrating capacity. Thus, the urinary composition of such a patient with prerenal azotemia who has received a diuretic may mimic that of a patient with AKI. There are a few other limitations to interpreting the urinary composition in differentiating AKI from prerenal azotemia. The urine of older patients and patients with chronic renal disease may not be concentrated despite the presence of prerenal azotemia. Fractional excretion of sodium (Fe_{Na}) is increased in ATN but may be low in association with AKI caused by nonoliguric ATN, radiocontrast, hepatorenal syndrome (HRS), rhabdomyolysis, acute glomerulonephritis, vasculitis, and early obstructive uropathy (559).

Table 10–9 Urine Findings in Prerenal Azotemia and "Intrinsic" or Parenchymal AKI

Laboratory test	Prerenal Azotemia	ATN
Urine sodium (U_{Na}), mEq/L	<20	>40
Urine osmolality, mOsm/kg H_2O	>500	<400
Urine to plasma urea nitrogen	>8	<3
Urine to plasma creatinine	>40	<20
Fractional excretion of filtered sodium	<1	>1
Urinary sediment	Bland	"Muddy" brown granular casts, cellular debris, tubular epithelial cells

AKI, acute kidney injury; ATN, acute tubular necrosis.

In a recent study, urine NGAL performed best at detecting AKI in patients entering the emergency room (560). In this study, patients with prerenal azotemia or CKD did not have increased urine NGAL. Fe_{Na}—but not urinary NGAL concentration—distinguished prerenal azotemia from CKD in this group of patients.

Finally, since urea clearances are flow dependent, the decreased urine flow and intact tubular function with prerenal azotemia or acute urinary tract obstruction are associated with reduced urea clearances. The rise in BUN, therefore, may be more rapid than the increase in serum creatinine concentration, since creatinine clearances are not flow dependent. In this regard, a ratio of BUN to serum creatinine concentration considerably >10 to 15:1 suggests prerenal azotemia or acute postrenal failure. With uncomplicated AKI, this ratio usually does not exceed 10 to 15:1. However, increased protein intake, blood in the gastrointestinal tract, or enhanced endogenous catabolic rate (e.g., fever, steroids, or trauma) may also increase the ratio of BUN to plasma creatinine. Alternatively, a low-protein diet or liver disease could lower the ratio of BUN to serum creatinine with prerenal or postrenal azotemia, or an increased catabolic rate could increase the ratio to >10 to 15:1 with AKI. Thus, as with evaluation of the composition of the urine, the interpretation of the ratio of BUN to plasma creatinine must be made with caution.

The relationship between urine microscopy findings at the time of nephrology consultation and clinical outcomes was evaluated prospectively (561). A urinary sediment scoring system was created in 249 patients on the basis of the number of renal tubular epithelial cells and granular casts. The urinary sediment combined scores were lowest in those with Stage 1 and highest in Stage 3 AKI. The urinary scoring system (a score of ≥3 vs. score of 0) was significantly associated with an increased risk of worsening AKI and was more predictive than AKI Network stage at the time of consultation. The study concluded that the urinary sediment score is a useful tool to predict worsening of AKI due to either ATN or prerenal AKI during hospitalization.

THE PATHOPHYSIOLOGY AND CLINICAL FEATURES OF THE COMMON CAUSES OF AKI

Nephrotoxins are an important cause of AKI. Some important nephrotoxins are aminoglycoside antibiotics, x-ray contrast media, NSAIDs, cisplatin, and amphotericin B.

Aminoglycoside Nephrotoxicity

Aminoglycosides are major antibiotics in the treatment of serious gram-negative infections. Their increased use and potential nephrotoxic risk have made them a frequent cause of AKI. AKI occurs in 10% to 25% of

patients on aminoglycosides even with careful dosing and therapeutic plasma levels. The nephrotoxicity of the aminoglycosides probably is related to their binding to the renal cortical tissue (562,563). Specifically, the binding at the apical surface of proximal tubule cells is now known to involve megalin. Once endocytosed, aminoglycosides inhibit endosomal fusion. They may also be directly trafficked to the Golgi apparatus (564). The tissue half-life of the aminoglycosides is much longer than that in serum; specifically, in the rat the half-life in serum of gentamicin has been shown to be 30 minutes, while in renal tissue it is 109 hours (565). The long tissue half-life explains why renal failure secondary to aminoglycosides can occur even after cessation of the antibiotics. A large body of in vitro and in vivo evidence indicates that reduced oxygen metabolites are important mediators of gentamicin nephrotoxicity (566). Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalysts can lead to the generation of hydroxyl radical. Gentamicin has been shown to release iron from renal cortical mitochondria and to enhance generation of hydroxyl radicals. These in vitro observations have been supported by in vivo studies in which scavengers of reactive oxygen metabolites and iron chelators have shown to be protective in gentamicin-induced AKI.

Table 10–10 Clinical Differences between Contract-Induced Nephropathy and Aminoglycoside Nephrotoxicity

Aminoglycoside Nephrotoxicity	CIN
Nonoliguric	Oliguric more than nonoliguric
Slow onset (days to weeks)	Fast onset (24 h)
Slow recovery	Faster recovery
Normal or high Fe_{Na}	Low Fe_{Na}

CIN, contract-induced nephropathy; Fe_{Na}, fractional excretion of sodium.

Several factors may predispose to aminoglycoside nephrotoxicity. These include advancing age, underlying renal disease, volume depletion, hypertension, and recent exposure to aminoglycosides or other nephrotoxic drugs. The clinical course of aminoglycoside nephrotoxicity is usually

gradual in onset and is related to the dose and duration of drug exposure. Frequently, mild proteinuria, lysozymuria, a defect in concentrating ability, and polyuria precede a decline in glomerular filtration. Early findings are isothenuria secondary to nephrogenic diabetes insipidus, magnesium, and potassium wasting. Later findings include azotemia. The AKI of aminoglycoside toxicity is characteristically nonoliguric and reversible with a low mortality. Frank azotemia secondary to aminoglycosides may develop for the first time after the drug has been discontinued; conversely, recovery of renal function following discontinuation of the nephrotoxic aminoglycoside is often delayed and may require weeks to months to be complete. The clinical differences between aminoglycoside nephrotoxicity and contrast-induced nephropathy (CIN) are shown in Table 10-10.

Contrast-Induced Nephropathy

Radiocontrast media-induced AKI has clinically different features compared to aminoglycoside toxicity. The essential clinical differences between CIN and aminoglycoside nephrotoxicity are shown in Table 10-10.

CIN has been recognized to be a cause of renal failure with increasing frequency in the past few years. The incidence of CIN is about 11.3% using the definition of a 25% increase in serum creatinine or an absolute increase in serum creatinine of 0.5 mg/dL (567). Radiocontrast agents cause AKI by causing renal vasoconstriction. Hypoxic tubular injury is important in the pathophysiology of radiocontrast nephropathy. Radiologic contrast agents markedly aggravate outer medullary physiologic hypoxia (568). Endothelins have been implicated in the pathophysiology of radiocontrast nephropathy (569). However, in a recent clinical study of 158 patients with chronic renal failure undergoing cardiac angiography, administration of a mixed ETA and ETB receptor antagonist with intravenous hydration resulted in an exacerbation of radiocontrast nephrotoxicity compared with hydration alone (570).

Predisposing factors include age (>55 years), prior renal insufficiency, diabetes mellitus with neurovascular complications, proteinuria, volume depletion, acute liver failure, and recent nephrotoxic drug exposure (571). Renal failure has been reported following a variety of arteriographic procedures as well as intravenous urography, computed tomography (CT) scan with contrast medium, cholangiography, and oral cholecystography (571). The onset of renal failure usually is abrupt within 24 hours after

exposure to contrast media and is characterized by oliguria, but it may be nonoliguric (572). Recovery of renal function generally occurs. However, patients with advanced renal failure, particularly diabetic patients with nephropathy, may never recover function following x-ray contrast-induced AKI and may require chronic hemodialysis (CHD) therapy. Newer nonionic agents may be less nephrotoxic in high-risk patients (573).

It can be minimized by hydration with saline before and after the contrast load (574). Diuretics should be avoided if possible. The hemodynamic effect of radiocontrast may be mediated by Ca^{2+}, since Ca^{2+} channel blockers attenuate the AKI. Recovery from radiocontrast-related AKI tends to begin within 2 to 3 days. Prophylactic oral administration of the antioxidant acetylcysteine, along with hydration, attenuates the reduction in renal function induced by contrast agents in patients with chronic renal insufficiency (575). However, in this study, a reduction in renal function was defined by a small increase in serum creatinine of at least 0.5 mg/dL, which did not result in morbidity or mortality. Also, the acetylcysteine-treated group actually had a decrease in serum creatinine compared with the baseline.

Clinical studies of the CCB, fenoldopam, to reduce CIN have been performed. A pooled analysis of five clinical trials comparing intravenous fenoldopam with saline/placebo/*N*-acetyl cysteine (NAC) for the prevention of CIN was performed (576). The risk ratio for the development of CIN in the fenoldopam group was 1.19 compared to the control group. This was not statistically significant suggesting that fenoldopam is no better than placebo/saline or NAC in preventing CIN.

Trials that compare intravenous sodium chloride to intravenous sodium bicarbonate for the prophylaxis of CIN have demonstrated conflicting results (577). Two recent meta-analyses have attempted to provide a more definitive answer to whether intravenous sodium chloride is better than intravenous sodium bicarbonate in preventing CIN (567,578). Both meta-analyses demonstrated that intravenous sodium bicarbonate is better in preventing CIN as defined by an increase in serum creatinine of 25% or an absolute increase in serum creatinine of 0.5 mg/dL. Intravenous sodium chloride was no different to intravenous sodium bicarbonate in preventing the need for RRT or death (567,578). The clinical trials of intravenous sodium chloride versus intravenous sodium bicarbonate performed to date in CIN have major limitations (577).

A meta-analysis was performed to analyze the efficacy of sodium bicarbonate in preventing CIN (579). Twenty randomized controlled trials (RCTs; n = 4,280) performed between 2004 and 2014 were analyzed.

Sodium bicarbonate is effective in preventing CIN among patients with preexisting renal insufficiency. However, it fails to lower the risks of dialysis and mortality and therefore may not improve the clinical prognosis of patients with CIN.

Preprocedural statin administration may reduce CIN. Eight RCTs comparing preprocedural statin administration before coronary catheterization with standard strategies were analyzed (580). The incidence of CIN was 3.91% in the statin group (n = 2,480) and 6.98% in the control group (n = 2,504). In the pooled analysis using a random-effects model, patients receiving statins had 46% lower relative risk of CI-AKI compared with the control group (P = 0.001). There was a benefit with statins in patients with GFR <60 mL/minute and a highly significant benefit in patients with GFR ≥60 mL/minute. Statin type and NAC or hydration did not significantly influence the results. The analysis concluded that preprocedural statin use leads to a significant reduction in the pooled RR of CI-AKI.

Adequately powered, well-designed clinical trials of interventions to prevent or treat CIN are urgently needed.

NSAIDs

NSAIDs, which are used in the management of pain and rheumatic disorders, are increasingly recognized as etiologic factors in AKI. These substances, which include a large group of newer nonsteroidal agents, COX-2 inhibitors, as well as aspirin and its derivatives, have in common the inhibition of prostaglandin synthesis (575). They have been incriminated in several renal abnormalities, including ischemic AKI (581), AIN (582), hyporeninemic hypoaldosteronism (583), papillary necrosis (584), and nephrotic syndrome (585).

Studies indicate that NSAID-induced AKI is due to the diminished renal vasodilatory effect of prostaglandins. In individuals receiving a 50-mEq sodium diet daily, indomethacin doses of 150 mg caused decrements in the GFR of <10% but no change in renal blood flow (586). However, chronic renal failure patients treated with the same indomethacin dose and diet regimen had more profound decrements in both the GFR and renal blood flow. Similar effects of diminished renal function and blood flow associated with the use of indomethacin and other NSAIDs have been reported in patients with lupus nephritis (587), nephrotic syndrome (588), cirrhosis with ascites (589), and severe congestive heart failure (590). Common to all of the edematous disorders is reduced effective arterial

circulating volume (decreased cardiac output or arterial vasodilatation) and renal vasoconstriction mediated by stimulation of sympathetic tone and the RAS. This renal vasoconstriction is normally attenuated by the vasodilatory effect of prostaglandins. Blocking prostaglandin synthesis with NSAIDs disrupts this balance, thus causing severe renal vasoconstriction and a reduced GFR. Other predisposing factors to NSAID-induced ischemic renal dysfunction include diuretic use, elderly age, atherosclerotic cardiovascular disease, renovascular disease, diabetes, and acute gouty arthritis (590). Patients with chronic renal insufficiency are also at risk of acute vasomotor decline in renal function with NSAIDs. Typical clinical features include the presence of risk factors, modest salt and water retention, decreased urinary output, a benign urine sediment, low Fe_{Na} (<1%), and prompt improvement in renal function on discontinuation of NSAIDs (581,583). Several of the NSAIDs have been associated with an acute allergic interstitial nephritis that is highlighted by renal failure, heavy proteinuria, and interstitial nephritis with foot process fusion (minimal change) on renal biopsy. This renal failure with NSAIDs is also generally reversible but in a slower fashion.

Cyclooxygenase metabolizes arachidonic acid into prostaglandin H_2. There are two isoforms of cyclooxygenase, designated COX1 and COX2. In the kidney, COX1 predominates in vascular smooth muscle and collecting ducts, whereas COX2 predominates in the macula densa and nearby cells in the cortical thick ascending limb. COX2 is also highly expressed in medullary interstitial cells (591). COX1 is constitutively expressed, while COX2 expression is typically low (592). COX2 expression is subject to regulation by salt intake, water intake, medullary tonicity, growth factors, cytokines, and adrenal steroids. Recently, COX2-selective NSAIDs have become widely available. Many of the adverse renal effects of nonselective NSAIDs appear to be mediated by the inhibition of COX2 rather than COX1. While COX2-selective NSAIDs spare the gastrointestinal tract, they appear to have the same adverse effects on the kidney as the nonselective NSAIDs. Clinical and experimental studies have shown that renal effects of COX2 inhibitors are similar to those of nonselective NSAIDs (593). These adverse effects include sodium, potassium, and water retention and decreases in renal function, as well as mild to modest increases in blood pressure and aggravation of edema. These adverse effects are potentiated in patients with volume and/or sodium depletion. Also, experimental studies showed increased renal COX2 expression in models of renal injury, including the remnant kidney, renovascular hypertension, diabetes, as well as during the

progression of renal failure (592). This suggests that COX2 inhibitors may confer a renoprotective effect in diverse renal disorders.

Cisplatin

Cisplatin is a very effective chemotherapeutic agent used in a number of tumor types. Despite routine use of hydration and mannitol, there is still a significant incidence of renal failure. Cisplatin nephrotoxicity is cumulative and dose dependent (594). A significant and transient increase in BUN and serum creatinine is observed in most patients after a single dose of 40 to 100 mg/m^2. At a high dose of 100 mg/m^2 given over 1 week, there is a prolonged renal failure that can last up to 2 years (595). Cisplatin causes tubular necrosis predominantly of S3 segments of proximal tubules (596). The decrease in renal plasma flow by cisplatin precedes the decrease in the GFR, suggesting that the primary effect of the drug in causing tubular necrosis may be due to decreased circulation in the vasa recta, causing damage to the adjacent S3 segments (597). In this regard, caspases and calpain are independent mediators of cisplatin-induced endothelial cell necrosis (146). Also the circulating von Willebrand factor (VWF), a measure of systemic endothelial injury, is increased in mice with cisplatin-induced AKI (372). However, cisplatin causes tubular cell death by apoptosis in cultured tubules, an in vitro system independent of renal vasculature (598,599). This suggests that cisplatin can also cause tubular injury directly. Cisplatin nephrotoxicity is also associated with urinary losses of magnesium, sodium, potassium, and Ca^{2+}, as well as hypomagnesemia and hypokalemia.

The pathogenesis of cisplatin-induced AKI has been widely studied in rodents (600). The antioxidant NAS (601) and the reducing agent glutathione (602) protect against cisplatin-induced AKI in rat models. Caspase-1-deficient mice are protected against cisplatin-induced ATN and tubular cell apoptosis (225). Cisplatin-induced ARF is associated with an increase in the cytokines IL-1β, IL-18, and IL-6 and neutrophil infiltration in the kidney (141). However, inhibition of IL-1β, IL-18, and IL-6 or neutrophil infiltration in the kidney is not sufficient to prevent cisplatin-induced ARF. Cisplatin-induced AKI in mice is associated with an increase in CD11b-positive macrophages in the kidney and increased expression of fractalkine (CX3CL1), a potent macrophage chemoattractant that is expressed on activated endothelial cells (372). However, macrophage depletion or fractalkine receptor (CX3CR1) inhibition is not sufficient to protect against the histologic and functional changes in

cisplatin-induced AKI. The pathophysiology of cisplatin-induced AKI has been reviewed in detail (600). The pathophysiology of cisplatin-induced AKI involves proximal tubular injury, oxidative stress, inflammation, and vascular injury in the kidney. There is predominantly ATN and also apoptosis and autophagy in the proximal tubules. There is activation of multiple proinflammatory cytokines and infiltration of inflammatory cells in the kidney. Inhibition of the proinflammatory cytokines TNF-α or IL-33 or depletion of mast cells protects against cisplatin-induced AKI. Cisplatin also causes endothelial cell injury.

Angiotensin-Converting Enzyme Inhibitors

Angiotensin-converting enzyme (ACE) inhibitors are cells widely used for the treatment of hypertension, congestive heart failure, and diabetic nephropathy. AKI may occur in conditions where angiotensin plays a crucial role in maintaining the GFR, such as volume depletion, bilateral renal artery stenosis, autosomal dominant polycystic kidney disease, cardiac failure, cirrhosis, and diabetic nephropathy. Diuretic-induced sodium depletion and underlying chronic renal insufficiency are major predisposing factors. Renal insufficiency is usually asymptomatic, nonoliguric, and associated with hyperkalemia. AKI is reversible in most cases after discontinuation of the ACE inhibitor.

Hepatorenal Syndrome

In cirrhosis of the liver, according to the systemic arterial vasodilation hypothesis, relative underfilling of the arterial tree triggers a neurohumoral response (activation of the renin–angiotensin–aldosterone system, SNS, nonosmotic release of vasopressin) aimed at restoring circulatory integrity by promoting renal sodium and water retention. Evidence has accumulated for a major role of increased vascular production of NO as the primary cause of arterial vasodilation in cirrhosis (383,603).

HRS should be considered in patients with decompensated cirrhosis with ascites presenting with AKI. This condition is typical of prerenal azotemia, as the urine sediment is bland and the kidney functions normally if transplanted into a person with a normal liver. It is a diagnosis of exclusion as other causes of AKI like ATN, hypovolemia, AIN, acute glomerulonephritis, and urinary obstruction need to be excluded. It has a 95% mortality rate but can be reversed by liver transplantation. Recent studies have shown that a V1 vasopressin antagonist, terlipressin, with

albumin for 7 to 10 days can reverse type 1 HRS (high risk for mortality within weeks) in approximately 50% of patients.

Recently, new diagnostic criteria for HRS have been proposed (604). The diagnosis of HRS is based on the exclusion of other causes of renal failure. The revised diagnostic criteria for HRS differ from the previously established criteria in that (a) renal failure in the setting of ongoing sepsis, but not septic shock, is considered as HRS; (b) plasma volume expansion should be performed with albumin rather than saline; and (c) minor diagnostic criteria like urine volume <500 mL/day, urine sodium <10 mEq/L, urine osmolality greater than plasma osmolality, and serum sodium concentration <130 mEq/L have been excluded. Major criteria that should be present to make a diagnosis of HRS are (a) cirrhosis with ascites, (b) serum creatinine >1.5 mg/dL, (c) absence of shock and no current or recent treatment with nephrotoxic drugs, (d) no sustained improvement of serum creatinine to a level <1.5 mg/dL following at least 2 days of diuretic withdrawal and volume expansion with albumin (1 g/kg/d up to a maximum of 100 g/day), and (e) absence of intrinsic kidney disease as indicated by proteinuria >500 mg/day, hematuria (>50 RBCs per high-power field), or abnormal renal ultrasound.

Atheroembolic Disease

Atheroembolic disease, sometimes separately designated as cholesterol crystal embolism, is an increasing and still underdiagnosed cause of renal dysfunction antemortem in elderly patients (605,606). A history of AKI occurring after cardiovascular surgery, angiography-induced aortic intimal trauma, or intravenous administration of streptokinase for myocardial infarction should raise a suspicion of atheroembolic disease as the cause of AKI. Occasionally it occurs spontaneously or in patients on Coumadin. There are showers of cholesterol crystals or microemboli from the surface of ulcerated plaques that travel distally to occlude small arterioles. Small emboli to the gut and pancreas may cause abdominal pain. Clinical examination may reveal peripheral vascular insufficiency, a “blue” toe, or livedo reticularis. Laboratory investigation may reveal an increased erythrocyte sedimentation rate, eosinophilia, and hypocomplementemia. The presentation and clinical findings can be confused with those of polyarteritis nodosa, allergic vasculitis, subacute bacterial endocarditis, or left atrial myxoma. The confirmatory diagnosis can be made by means of biopsy of the target organs, including kidneys, skin, and the gastrointestinal system. The renal outcome may be variable; some patients

deteriorate or remain on dialysis, some improve, and some remain with chronic renal impairment. Prevention of the disease involves avoiding unnecessary invasive procedures, for example, a renal arteriogram—manipulation of the aorta during surgery in patients with clinical evidence of widespread atherosclerosis. An aggressive therapeutic approach, for example, surgical bypass of infrarenal lesions with patient-tailored supportive measures, may be associated with a favorable clinical outcome (607).

Thrombotic Microangiopathies

Thrombotic microangiopathies are characterized by microangiopathic hemolytic anemia, thrombocytopenia, and variable renal and neurologic manifestations. This condition should be suspected in patients with AKI, thrombocytopenia, and neurologic signs like confusion and seizures. The causes of thrombotic microangiopathy and AKI are listed in Table 10-2. A peripheral blood smear will always show increased RBC fragmentation. The clotting profile, for example, international normalized ratio and partial thromboplastin time, is usually normal.

All of these disorders most likely begin with endothelial injury followed by secondary platelet thrombi formation in renal arterioles. Recent advances in the pathophysiology of thrombotic thrombocytopenic purpura (TTP) and HUS are the newly discovered VWF, multimer-cleaving protease, endothelial cell apoptosis induced by serum from patients with TTP, and atypical HUS and the activation of the complement system (608). Renal cortical necrosis may result from arterial lesions. When acute azotemia develops in association with these disorders, it often represents only one of many serious complications of an underlying disease. Moreover, the primary site of injury is the glomerulus or the vascular supply of the glomerulus, with the proximal tubule and the interstitial areas relatively uninvolved. Recovery of renal function following HUS in children is expected. Plasmapheresis is the treatment of choice in TTP.

Therapies under development for TTP include recombinant ADAMTS13 in patients with hereditary TTP and anti-VWF (caplacizumab) that blocks the VWF–platelet interaction.

Caplacizumab is a humanized monoclonal antibody-based fragment (a nanobody) that binds to VWF and blocks VWF interaction with platelet GPlb-IX-V.

Eculizumab, a humanized monoclonal antibody to C5, has been used in

patients with severe complement-mediated HUS who are at risk of death or ESRD. Prophylactic administration of eculizumab is also recommended to prevent recurrent disease in the allograft for renal transplant recipients with an identified mutation in complement factors, CFH, CFI, C3, or CFB, or in those with a previous posttransplant episode of recurrent disease. However, because of the prohibitive cost of eculizumab, TPE is an alternative therapy in these patients.

Acute Uric Acid Nephropathy

Acute uric acid nephropathy causes AKI due to intratubular deposition of uric acid crystals. There is a very high serum uric acid concentration. It typically occurs during induction chemotherapy for malignancies with high cell turnover, for example, leukemias and lymphoproliferative malignancies. Acute uric acid nephropathy and AKI occur in the tumor lysis syndrome. Clinical features of acute uric acid nephropathy are hyperuricemia, hyperkalemia, hyperphosphatemia, and a urine urate:creatinine ratio >2. Preventive measures include vigorous hydration and alkaline diuresis. Allopurinol should be started several days before the chemotherapy.

Acute Phosphate Nephropathy

AKI has been reported after the administration of oral sodium phosphate solution as bowel preparation for colonoscopy (609). A common bowel-cleaning regimen is 45 mL of oral sodium phosphate solution containing 21.6 g of monobasic sodium phosphate and 8.1 g of dibasic sodium phosphate, which is equal to 5.8 g of elemental phosphorus. Five criteria have been proposed to make a diagnosis of acute phosphate nephropathy (609): (a) AKI, (b) recent exposure to oral phosphate, (c) renal biopsy findings of acute and chronic tubular injury with abundant calcium phosphate deposits (usually involving >40 tubular lumina in a single biopsy), (d) no evidence of hypercalcemia, and (e) no other significant pattern of renal injury on renal biopsy. Risk factors for the development of acute phosphate nephropathy include preexisting CKD, inadequate hydration, older age, hypertension treated with ACE inhibitors, ARBs or loop diuretics, female gender, and NSAIDs. Oral phosphate solution is contraindicated in patients with CKD, congestive heart failure, gastrointestinal obstruction, and preexisting electrolyte disorders like hypercalcemia. In animal studies, it remains controversial whether acute

phosphate administration alone can cause AKI in the absence of kidney injury from other causes, for example, hypotension and NSAIDs. Female rats fed purified diets containing either 0.4% or 0.6% (wt/wt) phosphorus for 28 days develop nephrocalcinosis and renal impairment (610). However, in another study, rats fed oral phosphate did not get renal failure or nephrocalcinosis (611).

Melamine Toxicity

Melamine is an organic nitrogenous compound used commercially in the production of various products like plastics. Recently, in China, melamine was added to milk to falsely elevate protein assay results (612). This resulted in melamine toxicity mostly in children, causing kidney stones due to melamine/cyanuric acid crystals, chronic inflammation in the kidneys, and AKI. An outbreak of AKI in dogs and cats was related to melamine in pet foods (613).

Acute Interstitial Nephritis

AIN is a disorder characterized by acute renal insufficiency usually due to infection or drug exposure. In the kidney, the pathologic changes include an acute interstitial inflammatory exudate and edema. The pathology as seen on renal biopsy is similar regardless of the etiology. There is interstitial edema with variable numbers of polymorphonuclear leukocytes, eosinophils, mononuclear cells, and plasma cells. The glomeruli appear normal, and the tubules show abnormalities that include necrosis, degeneration, or atrophy. The distribution of the tubular changes is patchy.

The earliest cases of this disease were detected in association with diphtheria, syphilis, and streptococcal and other bacterial infections (614). More recently, AIN has been diagnosed in leptospirosis (615), Legionnaires disease, infectious mononucleosis, and falciparum malaria (616). Other protozoan, fungal, and rickettsial agents have also been causally incriminated in AIN. Infection-related AIN usually presents as renal failure complicating the underlying disease.

There are arguments to suggest that drug-induced AIN is secondary to an immune reaction in humans (617). It only occurs in a small proportion of people taking the drug. It is not dose dependent. It is sometimes associated with extrarenal manifestations of hypersensitivity and it recurs after reexposure to the drug or a closely related drug.

Endogenous renal antigens can induce AIN. In a rat model, antisera to

THP caused in situ granular immune complexes in the ascending limb of the loop of Henle (618). In another animal model, Brown–Norway rats have been found to develop antitubular basement membrane antibodies and tubulointerstitial nephritis when injected with homologous tubular basement membrane (619). Renal mononuclear cell infiltration has also been shown in rats injected with homologous or heterologous kidney preparations, suggesting a cell-mediated inflammatory response to autologous antigens (620). Other studies in rats (621) identified activated and immunologically suppressible T cells in inflammatory kidney infiltrates, which also suggests a cell-mediated immunologic response in interstitial nephritis. Human counterparts of these animal studies have been suggested by a number of investigators. Tubular immune complexes have been demonstrated in 50% of lupus nephritis patients (622). Interstitial inflammatory infiltrates are frequently found in association with tubular deposits. Antitubular basement membrane antibodies have been detected in patients with antiglomerular basement membrane-mediated disorders such as Goodpasture syndrome (622). Antitubular basement membrane antibodies have also been found in renal allografts and poststreptococcal glomerulonephritis (622). In antiglomerular basement membrane disease (622), evidence exists that cell-mediated immunity develops against renal antigens. Interstitial lymphocyte infiltrates are frequently seen in this disorder.

Experimental AIN can also be induced by promoting immune reactions against extrarenal proteins that become trapped in the kidney (“planted” antigens) (617). AIN can be induced by injecting rabbits with bovine serum albumin or by injecting aggregated bovine gamma globulins into the kidney of presensitized animals (623).

In animal models of AIN there is either cell-mediated or antibody-mediated immunity (624). However, in humans, AIN probably involves cell-mediated immunity, as immune deposits are not usually seen on renal biopsy. Also, interstitial infiltrates consist of T cells.

Experimental studies show that macrophages, lymphocytes, and activated tubular cells in vitro can produce cytokines that result in proliferation of fibroblasts and/or increase in extracellular matrix (625). The agents produced by these inflammatory cells include TGF-β, IL-1, IL-4, IGF-1, ET-1, and lipid peroxidation products. Accumulation of extracellular matrix may lead to permanent impairment of renal function and the antiinflammatory TGF-β may induce interstitial fibrosis.

Most drug-related reports of AIN have been with the use of penicillin and, in particular, its synthetic analogs such as methicillin. The antibiotic-

induced acute allergic interstitial nephritis may have the clinical findings of a hypersensitivity reaction, with fever, rash, joint pain, and eosinophilia. The urinary sediment is not diagnostic, showing mild proteinuria (<1.5 g/day), pyuria, hematuria, and granular casts. Urine cultures are usually negative. Other urinary findings include impaired concentrating ability, urinary acidification, as well as decreasing potassium excretion. Renal failure is variable. Drug-related AIN also may have a paucity of clinical findings. There may be raised IgE levels in the serum of patients with drug-induced interstitial nephritis.

Recently, there has been considerable interest in the role of NSAIDs in acute allergic interstitial nephritis. The interstitial disorder associated with NSAIDs can occur separately from the ischemic injury and often presents with nephrotic range proteinuria in the absence of clinical findings of a hypersensitivity reaction. However, acute deterioration of renal function may be the only manifestation. Proton pump inhibitors are an increasingly recognized cause of AIN and may even be associated with CKD (626). The clinical features of the two major forms of acute allergic interstitial nephritis are listed in Table 10-11.

In general, recovery occurs with treatment of the underlying disease or removal of the offending drug. However, there have been reports of permanent impairment of renal function or death (616). There is some indication that heavy proteinuria in the nephrotic range and renal granulomas on biopsy are associated with a poor outcome. The use of steroid therapy is controversial, since there is no large randomized prospective controlled study indicating a beneficial effect of steroids (617). However, a brief course of corticosteroids can hasten the recovery of renal function (617). Despite the lack of scientific evidence, a short course of prednisone in a patient whose renal function fails to improve within 1 week of stopping the inciting drug is recommended, provided the diagnosis of AIN is confirmed by renal biopsy (617).

Table 10–11 Two Major Forms of Acute Allergic Interstitial Nephritis

	β-Lactam Antibiotics	NSAIDs
	Any age	Older than 60
	Days	Months
	80%	20%
	<1 g/day	Nephrotic

Age	20%	40%
Duration of therapy	Methicillin	Fenoprofen
Fever, rash, eosinophilia/uria	β-Lactam antibiotics	Aspirin
	Ciprofloxacin	Ibuprofen
Proteinuria	Sulfonamides	Indomethacin
Requirement for dialysis	Erythromycin	Naproxen
Commonest agent	Rifampicin	Phenylbutazone
Other common agents	Phenytoin	Piroxicam
	Furosemide	Tolmetin
	Allopurinol	Zomepirac
	Cimetadine	Any NSAID
	PPIs, e.g., omeprazole	—

NSAID, nonsteroidal antiinflammatory drug, PPI, proton pump inhibitor.

AKI in Patients with Acquired Immunodeficiency Syndrome

The approach to the causes of AKI in AIDS patients is the same as that for other patients, that is, prerenal, intrinsic renal, and postrenal causes. Causes of AKI that are especially associated with human immunodeficiency virus infection are given in Table 10-12. AKI may develop in 6% to 20% of hospitalized patients with AIDS and is in most cases multifactorial (627). In a study of 449 patients admitted to a hospital in New York City, the causes of AKI were hypovolemia (38%), drug toxicity (37%), ATN from shock or sepsis (8%), and radiocontrast nephropathy (4%) (628). AKI secondary to tenofovir nephrotoxicity has recently been described (629). A distinct form of AIN secondary to diffuse infiltrative lymphocytosis syndrome has also been reported in AIDS patients (629) (Table 10-12).

Table 10–12 AKI in AIDS Patients

Prerenal
Hypovolemia (diarrhea)
Hypotension (sepsis, bleeding)
Vasoconstriction (radiocontrast agents)
Renal
ATN (shock, bacteremia, aminoglycosides, amphotericin, tenofovir)
Rhabdomyolysis (pentamidine, zidovudine, didanosine)
Acute allergic interstitial nephritis (penicillins, sulfonamides)

Postinfectious glomerulonephritis Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura Diffuse infiltrative lymphocytosis syndrome
Postrenal
Tubular obstruction due to crystalluria (intravenous acyclovir, sulfadiazine, indinavir, saquinavir, ritonavir) Extrinsic ureteral compression (lymph nodes, tumors) Intrinsic ureteral obstruction (fungus balls) Bladder obstruction (tumors, fungus balls)

AKI, acute kidney injury; AIDS, acquired immunodeficiency virus; ATN, acute tubular necrosis.

New antiviral agents for the treatment of AIDS have led to increased survival and improved quality of life. Protease inhibitors are important drugs in patients with AIDS. AKI is a rare but important complication of indinavir treatment (630). The renal function of patients receiving indinavir should be closely monitored. Benign and asymptomatic crystalluria occurs in 4% to 13% of AIDS patients receiving indinavir. Interstitial nephritis has also been reported. A hydration protocol consisting of 1 to 2 L of fluid should be initiated 3 hours after each indinavir dose. If AKI persists, temporary indinavir withdrawal or switching to another protease inhibitor should be considered.

Importantly it should be realized that ATN in AIDS patients may be avoidable in some cases when preventive measures are used, for example, maintaining adequate hydration before use of radiocontrast agents and during use of antibiotics and antiretroviral therapy that precipitates crystalluria (631).

Management of AKI

FLUID AND ELECTROLYTES

The use of dialysis therapy for oliguric AKI may permit fluid intakes of 1.5 to 2.0 L/day, depending on the volume status of the patient. If dialysis is not immediately available, fluid balance usually can be maintained by replacing insensible losses (400–600 mL/day) with 10% dextrose in water and measured losses (e.g., urine, gastric drainage, diarrhea) liter for liter with 0.45% saline. The best way to monitor the adequacy of fluid therapy

is a clinical examination of ECF volume status, urine output, and daily weights. A serum sodium determination is useful in deciding whether water intake is appropriate to solute intake. Hyponatremia generally indicates excessive water intake, and hypernatremia indicates too little water intake. Since hypokalemia is rarely a problem in patients with oliguric AKI, and because of the dangers of hyperkalemia, potassium chloride is not added to intravenous fluids.

In general, fluid management in nonoliguric AKI tends to be easier. These patients ordinarily should receive a volume of fluid per day that equals their urine output plus insensible losses. The salt content of their diets should be approximately equal to what is excreted in the urine and lost in other measurable bodily fluids.

The type of fluid to use in resuscitation of AKI patients has been a topic of intensive study. A comprehensive Cochrane review concluded that there is no evidence from randomized clinical trials that resuscitation with colloids, instead of crystalloids, reduces the risk of death in patients with trauma, burns, or following surgery (632). Recent large trials and meta-analyses suggest no mortality benefit and possible harm with hydroxyethyl starch use for resuscitation of critically ill patients (633).

DIURETICS

The use of diuretic agents in the treatment of AKI has several theoretical benefits. As tubular obstruction by casts is thought to contribute to the pathophysiology of AKI, prevention of cast formation might be protective. Osmotic diuretics in addition to increasing mean arterial pressure could serve to augment tubular flow and "flush out" obstructed tubules. Loop diuretics could similarly increase flow and, by inhibiting the NaK_2Cl transporter in the thick ascending limb of the loop of Henle, decrease medullary oxygen demand (634). Additionally, it has been observed that nonoliguric patients fare better than oliguric patients (553). While conversion of oliguria to nonoliguria has not been shown to decrease mortality, it may facilitate fluid and electrolyte management in patients with AKI. However, studies show that diuretics do not improve mortality in AKI and should not be used in the absence of hypervolemia (635).

In the setting of prevention of myoglobinuric-related AKI, mannitol is widely used. Prospective randomized controlled data, however, are lacking. There have been reports of patients with rhabdomyolysis treated with mannitol (636,637) with the suggestion that when aggressive hydration is begun early following muscle injury, AKI can be prevented

(638). Mannitol coupled with bicarbonate diuresis is commonly prescribed in rhabdomyolysis (639).

While loop diuretics have been used to facilitate fluid management in AKI, they do not attenuate the course of illness or improve mortality (588,640–642). Also, in these studies, deafness as a complication of high-dose loop diuretics has been reported. In a study of 552 patients with AKI in the ICU setting, the use of diuretics was associated with an increased risk of death and nonrecovery of renal function (643). It was concluded that it is unlikely that diuretics confer benefit in AKI patients in the ICU.

NUTRITIONAL SUPPORT

AKI in the setting of multiorgan failure is a state of metabolic stress (644). Catabolism of protein stores to support gluconeogenesis can result in marked muscle and visceral protein wasting and is associated with excess morbidity and mortality.

While the benefits of nutritional support of critically ill patients with AKI are unproven, enteral compared with parenteral feeding may be of benefit. In a study of 75 patients with abdominal trauma undergoing laparotomy, enteral nutrition was associated with improved nutritional markers and decreased infectious complications and sepsis compared with parenteral nutrition (645).

However, in the setting of AKI, parenteral nutrition has not been proven to be of benefit. With multiorgan dysfunction, uremia is known to accelerate catabolism due to a variety of factors, including acidosis, altered counterregulatory hormonal status, increase in plasma protease activity, and insulin resistance. A prospective double-blind study randomizing 30 patients with AKI to three isocaloric regimens—glucose alone, glucose plus essential amino acids, and glucose plus essential and nonessential amino acids—has been performed (646). All patients remained in negative nitrogen balance throughout the study, and no difference in recovery of renal function or of survival between treatment groups was noted. In patients on CRRT, despite an intake of 2.5 g/kg/d of protein, these patients remained in negative nitrogen balance (647).

In recent reviews of the topic, the following recommendations were made (648,649): (a) protein and nonprotein calories should be provided to meet calculated energy expenditures and at a rate not to exceed 1.5 g/kg/d protein intake; (b) nutritional recommendations should not be different from that of critically ill patients as a whole; (c) total parenteral nutrition should be administered only to patients who are severely malnourished or

patients expected to be unable to eat for >14 days; and (d) enteral feeding is the preferred means of nutritional supplementation.

Specific Therapies for AKI

Although the mortality in patients with AKI has declined between 1988 and 2002 (6), the mortality of AKI in the ICU remains high (1). Most interventional therapeutic trials in AKI, for example, furosemide (642), dopamine and furosemide (650), anaratide (354,355), IGF-1 (488), and fenoldopam (651) have failed in humans. A possible reason for the failure of interventional trials in AKI is the dependence on serum creatinine to diagnose AKI. Alterations in serum creatinine may lag 24 to 48 hours behind actual changes in the GFR (652,653). Ideally, in the future, early diagnosis of AKI using urine or plasma biomarkers may allow early initiation of specific therapies, for example, EPO to treat or prevent worsening of AKI.

DIALYSIS

The various complications of AKI are listed in Table 10-13. The presence of severe hyponatremia may mimic or accentuate symptoms of uremia, and hyperkalemia may lead to severe cardiac disturbances. Symptomatic hypermagnesemia probably occurs only when patients with AKI are treated with magnesium-containing antacids. Hyperuricemia of moderate degree (10–14 mg/dL) is a frequent accompaniment of AKI, but the occurrence of gouty arthritis is very rare. In severely catabolic states associated with muscle breakdown, for example, rhabdomyolysis, the level of hyperuricemia may be substantially greater. Fluid overload is generally primarily responsible for the occurrence of hypertension and cardiac failure in AKI, and removal of fluid by dialysis is the most appropriate treatment. Gastrointestinal and neurologic symptoms and hemorrhagic disorders of the uremic patient with AKI should be immediately treated with dialysis. The anemia of AKI may occur more rapidly than expected from bone marrow suppression of erythropoiesis, and thus, in contrast to chronic renal failure, hemolysis may have a predominant role. However, the anemia generally does not necessitate treatment with transfusions unless simultaneous blood loss occurs. Infections remain the main cause of death in patients with AKI despite the vigorous use of dialysis. Thus, meticulous aseptic care of intravenous catheters and wounds and

avoidance of the use of an indwelling urinary catheter are important in the management of such patients. Fluid overload, which leads to hypoxia, and mechanical ventilation increase mortality and, if possible, should be avoided.

Table 10–13 Complications of AKI

Metabolic
Hyponatremia
Hyperkalemia
Hypocalcemia, hyperphosphatemia
Hypermagnesemia
Hyperuricemia
Cardiovascular
Pulmonary edema
Arrhythmias
Hypertension
Pericarditis
Neurologic
Asterixis
Neuromuscular irritability
Myoclonus
Somnolence
Seizures
Coma
Hematologic
Anemia
Bleeding
Gastrointestinal
Nausea
Vomiting
Bleeding
Infectious
Pneumonia
Bacteremia, e.g., secondary to dialysis catheter infection
Wound infection

AKI, acute kidney injury.

The main considerations when starting a patient with AKI on dialysis are the following: (a) initiation of dialysis, (b) dose of dialysis, (c) modality of dialysis, and (d) type of dialysis membrane.

Initiation of Dialysis

The indications for starting dialysis in AKI are not specific and may differ in individual patients. Guidelines are given in Table 10-14 (654). The medical records of 100 adult trauma patients treated with CRRT for posttraumatic ARF were retrospectively reviewed (655). Patients were characterized as "early" or "late" starters, on the basis of BUN < or >60 mg/dL, before CRRT initiation. Survival was 39% in early starters compared with 20% in late starters ($P = 0.041$). In a study of 106 critically ill patients with oliguric ARF, survival at 28 days and recovery of renal function were not improved by early (within 12 hours of a creatinine clearance <20 mL/minute) initiation of continuous venovenous hemofiltration (CVVH). In 243 patients from the Program to Improve Care in Acute Renal Disease (PICARD) study, the risk of death was determined in patients with BUN > or <76 mg/dL at the initiation of dialysis. After adjustment for age, hepatic failure, sepsis, thrombocytopenia, and serum creatinine and stratified by site and initial dialysis modality, the RR for death was 1.85 (95% confidence interval [CI] 1.16–2.96) for the patients with BUN >76 mg/dL (656). This study provides the rationale for prospective trials of the early initiation of RRT in AKI patients.

A systematic review and meta-analysis of RCTs and cohort comparative studies to assess the effect of "early" initiation of RRT versus "late" initiation of RRT on mortality in patients with AKI was performed (657). The primary outcome measure was the effect of early RRT on mortality stratified by study design. Twenty-three studies (5 randomized or quasi-RCTs, 1 prospective and 16 retrospective comparative cohort studies, and 1 single-arm study with a historic control group) were analyzed. In randomized trials, early RRT was associated with a nonsignificant 36% mortality risk reduction (RR, 0.64; 95% CI, 0.40–1.05; $P = 0.08$). Conversely, in cohort studies, early RRT was associated with a statistically significant 28% mortality risk reduction (RR, 0.72; 95% CI, 0.64–0.82; $P < 0.001$). This meta-analysis suggests that early initiation of RRT in patients with AKI might be associated with improved survival

(657).

Table 10–14 Guidelines for Initiation of Dialysis in AKI (654)

1. Oliguria (<400 mL/day)
2. Anuria
3. Serum creatinine >6−7 mg/dL
4. Plasma urea >80−100 mg/dL
5. Pulmonary edema unresponsive to conservative therapy
6. Hyperkalemia (serum potassium >6.5 mEq/dL)
7. Symptomatic uremia, i.e., encephalopathy, pericarditis
8. Metabolic acidosis

One criterion = grounds to start dialysis. More than one criterion = mandatory to start dialysis.
AKI, acute kidney injury.

In the multicenter Artificial Kidney Initiation in Kidney Injury (AKIKI) trial, 620 patients with KDIGO Stage 3 AKI who required mechanical ventilation, vasopressor therapy, or both but did not have life-threatening complications requiring immediate RRT, were studied (658). Patients were randomly assigned to either immediate RRT (early strategy) or delayed RRT in which such therapy was initiated if patients had development of severe hyperkalemia, uremia, metabolic acidosis, pulmonary edema, or severe oliguria that persisted for more than 72 hours after randomization. The primary outcome, mortality at 60 days, was similar in the two groups (48.5% in the early-strategy group and 49.7% in the delayed-strategy group, $P = 0.79$). However, an important finding in the study was that 49% of the patients in the delayed-strategy group never received dialysis, highlighting the need for dynamic risk stratification tools to identify patients who will not need RRT for management of their AKI (659).

Dose of Dialysis

There have been studies evaluating the dose of dialysis in AKI. In a small prospective study of Vietnam soldiers with ATN due to trauma, patients were assigned to intensive dialysis to keep predialysis serum creatinine <5 mg/dL and BUN <70 mg/dL or conventional dialysis to keep serum creatinine <10 mg/dL and BUN <150 mg/dL (660). Mortality was reduced with intensive versus conventional therapy, 3 of 8 patients dying (36%)

versus 8 of 10 patients dying (80%). In a prospective paired study in 34 civilian patients with AKI, patients were assigned to intensive dialysis (predialysis BUN <60 mg/dL and serum creatinine <5 mg/dL) or conventional dialysis (BUN <100 mg/dL and creatinine <9 mg/dL). Intensive dialysis resulted in a decrease in hemorrhagic events, and the mortality was 58.8% in the intensive group and 47.1% in the conventional group, which was not statistically significant (661). In a more recent study, an inverse relationship was found between the delivered dose of dialysis and patient survival (662).

The *KT/V* is a dimensionless index that takes into account the urea clearance rate, *K*, the time on dialysis, *T*, and the size of the urea pool, *V*, and is calculated for an individual hemodialysis therapy (663). The measurement of the delivered dose of dialysis with *KT/V*, which was designed for use in chronic renal failure, has recently been tested in AKI in a study of 46 dialysis treatments in 28 consecutive patients (664). Blood-based kinetics used to estimate the dose of dialysis in AKI patients on IHD provided internally consistent results. However, when compared with dialysate-side kinetics, blood-based kinetics substantially overestimated the amount of solute (urea) removal. In another study in AKI, nearly 70% of the treatments delivered a *KT/V* <1.2, the minimally acceptable dose defined in the Dialysis Outcomes Quality Initiative (DOQI) guidelines for CHD patients (665).

In recent trials employing biocompatible membranes in CRRT, there has been a suggestion that an increased dialysis dose was beneficial. The effect of the delivered dose of dialysis on mortality among different strata of a novel scoring system for critical ill AKI patients was examined (666). Among the patients with the lowest and the highest scores, the delivered dose of dialysis had no effect on mortality. Among patients at intermediate risk for death, a higher dose of dialysis (e.g., >58% urea reduction ratio) was associated with improved survival. As the delivered *KT/V* in these studies was observed and was not assigned by random allocation, the significance of these findings remains in question. For example, the patients who achieved a higher *KT/V* may have been able to tolerate hemodialysis better than the other patients, thereby introducing bias into the analysis. Thus, the optimal *KT/V* in AKI is not known (667).

Table 10–15 Dose of Dialysis in AKI

Study	Results	References

425 patients; single center; dose of CVVH	Ultrafiltration at 35 or 45 mL/h/kg, better patient survival than 20 mL/h/kg	(668)
160 patients; single center; daily or alternate-day hemodialysis	Daily hemodialysis, better patient survival than alternate-day hemodialysis	(669)
206 patients; single center; increase in dialysis dose by adding CVVHDF to CVVH	CVVH + CVVHDF, better patient survival than CVVH alone	(670)
ATN study; 1,124 patients; multicenter; intensive vs. conventional dose of dialysis	Patient survival the same, intensive dialysis vs. conventional dialysis	(671)
RENAL study; 1,508 patients; effect of increased dialysis dose on survival	Patient survival the same, intensive dialysis vs. conventional dialysis	(672,673)

AKI, acute kidney injury; CVVH, continuous venovenous hemofiltration; CVVHDF, continuous venovenous hemodiafiltration; ATN, acute tubular necrosis; RENAL, Randomized Evaluation of Normal versus Augmented Level Replacement Therapy.

Three recent single-center studies have demonstrated that an increased dose of dialysis is associated with lower mortality. A prospective randomized study of the impact of different ultrafiltration doses in CRRT on survival has recently been performed (668) on 425 patients, with a mean age of 61 years, in intensive care, who had AKI. The patients were randomly assigned ultrafiltration at 20 mL/h/kg (group 1, n = 146), 35 mL/h/kg (group 2, n = 139), or 45 mL/h/kg (group 3, n = 140). The primary end point was survival at 15 days after stopping hemofiltration. Survivors in all groups had lower concentrations of BUN before continuous hemofiltration was started than nonsurvivors. The frequency of complications was similarly low in all groups. Mortality among these critically ill patients was high, but patients receiving the higher dose of ultrafiltration (groups 2 and 3) had significantly improved survival. In another study, 160 patients with ARF were assigned to receive daily or alternate-day IHD. The mortality rate, according to the intention-to-treat analysis, was 28% for daily dialysis and 46% for alternate-day dialysis (P = 0.01) (669). The hypothesis that an increase in dialysis dose obtained by using continuous venovenous hemodiafiltration (CVVHDF) is associated with better survival than CVVH was tested in 206 patients (670). Twenty-eight-day and 3-month survival was significantly higher in the CVVHDF

group that received an increased dose of dialysis.

In view of the promising single-center studies, a large multicenter study, the ATN study, was performed by the Veterans Administration/National Institutes of Health ARF Trial network (671). In 1,124 critically ill patients with AKI, intensive renal support did not decrease mortality, improve recovery of kidney function, or reduce the rate of nonrenal organ failure compared with less-intensive therapy.

The Randomized Evaluation of Normal versus Augmented Level of Replacement Therapy (RENAL) study in over 1,500 patients is the largest interventional trial in AKI patients (672,673). In the RENAL study, 1,508 critically ill adults with AKI were randomized to CRRT in the form of CVVH with an effluent flow of either 40 mL/kg of body weight per hour (higher intensity) versus CVVH with a flow rate of 25 mL/kg/h (lower intensity) (673). At 90 days after randomization, the mortality was 44.7% in each group (odds ratio, 1.00; 95% CI, 0.81–1.23; $P = 0.99$). At 90 days, 6.8% of survivors in the higher intensity group (27 of 399), as compared with 4.4% of survivors in the lower intensity group (18 of 411), were still receiving RRT (odds ratio, 1.59; 95% CI, 0.86–2.92; $P = 0.14$). The study concluded that in critically ill patients with AKI, treatment with higher intensity CRRT did not reduce mortality at 90 days (673). The studies examining the dose of dialysis in AKI are listed in Table 10-15.

Modality of Dialysis

In critically ill patients with AKI in the ICU, either a continuous or intermittent modality of dialysis is chosen. In IHD, the patient is connected to a dialysis machine for 2 to 5 hours at a time daily or every second day. Rapid removal of solutes and fluids leads to peaks and troughs in BUN and serum creatinine and hemodynamic instability. Daily treatment for 4 hours, with a blood urea clearance of 200 mL/minute, can achieve a weekly urea clearance of 350 L (674). In CRRT, the patient undergoes continuous dialysis for 24 hours a day. There is a slow, continuous, and more gradual removal of solutes and fluid. This may allow massive fluid removal and remove some proinflammatory cytokines. Disequilibrium and hemodynamic instability caused by rapid solute and fluid removal are avoided. Minimization of hypotension theoretically avoids the perpetuation of renal injury. The technique, however, requires immobilization and continuous anticoagulation. The most widely used CRRTs are CVVH and continuous venovenous hemodialysis (CVVHD). CVVHD can achieve a weekly urea clearance of 340 L by use of any

combination of ultrafiltration rate and dialysate flow rate that add up to 2 L/hour (674).

Several retrospective and prospective studies have attempted to compare outcomes for continuous versus intermittent modalities. In a retrospective study of 349 patients, the mortality rate was higher for continuous versus intermittent dialysis (68% vs. 41%, $P < 0.001$) (675). However when multivariate cox analysis was employed to adjust for reasons for patient assignment to continuous treatment (e.g., systolic blood pressure <90 mm Hg, liver failure, etc.), there was no increase in risk of death with continuous treatment. In another prospective study, 225 patients in the ICU were divided into three groups: group I (control group), 156 patients with AKI who did not receive dialysis; group II, 21 patients who received IHD or peritoneal dialysis; and group III, 43 patients who received continuous hemodiafiltration. The mortality was higher in patients with renal failure who required dialysis. There was no difference in mortality between patients who required IHD versus CRRT (676).

A multicenter, randomized, controlled trial was conducted comparing IHD and continuous hemodiafiltration in the ICU (677). One hundred sixty-six patients were randomized. Principal outcome measures were ICU and hospital mortality, length of stay, and recovery of renal function. Despite randomization, there were significant differences between the groups in several covariates independently associated with mortality, including gender, hepatic failure, APACHE II and III scores, and the number of failed organ systems, in each instance biased in favor of the intermittent dialysis group. Using logistic regression to adjust for the imbalances in group assignment, the odds of death associated with continuous therapy was 1.3 (95% CI, 0.6–2.7, P = NS vs. IHD). In the most recent study of intermittent versus continuous dialysis in 316 critically ill patients, the modality of RRT had no effect on the outcome (678). In a meta-analysis of 1,635 patients from nine randomized control trials, it was concluded that CRRT does not confer a survival advantage over IHD (679).

A new hybrid technique named sustained low-efficiency dialysis (SLED), in which standard IHD equipment is used with reduced dialysate and blood flow rates, has recently been described in a single-center study (680). Twelve-hour treatments were performed nocturnally, allowing unrestricted access to the patient for daytime procedures and tests. One hundred forty-five SLED treatments were performed in 37 critically ill patients in whom IHD had failed or been withheld. The study concluded that SLED is a viable alternative to traditional CRRTs for critically ill

patients in whom IHD has failed due to hypotension or been withheld (680).

The difficulties in designing trials comparing intermittent with continuous modalities and also in AKI in general were recently reviewed (653). Prospective randomized studies are difficult to do, since hemodynamically unstable patients or patients who cannot tolerate IHD will almost always be started on CRRT. In patients with liver and renal failure, CRRT is the treatment of choice (681). Alternately, it may be unethical to confine a mobile patient to bed to receive CVVHD. Thus randomization may be biased. CRRT therefore may be considered the modality of choice in very ill patients, while IHD is used in less ill patients. At present, IHD and CRRT are regarded as equivalent methods for the treatment of AKI (653). The choice of IHD or CRRT should be made in consultation with a nephrologist and tailored for the individual patient. The decision may also depend on facility-specific issues like experience, nursing resources, and technical proficiency. The decision with respect to the mode of dialysis must be an individual one. For example, a severely catabolic patient with trauma, fever, or rhabdomyolysis or following an operation will present initially with high BUN. Aggressive and even daily treatment with hemodialysis therefore is indicated for this group of patients.

TYPE OF DIALYSIS MEMBRANE

Interaction between blood and artificial membranes may cause adverse effects. Adverse effects of bioincompatible membranes, for example, cellulose, cuprophane, hemophane, and cellulose acetate, include activation of complement and hypotension. Biocompatible membranes are made of synthetic polymers and include polyamides, polycarbonate, and polysulfone. Synthetic membranes are regarded as being more “biocompatible” in that they incite less of an immune response than cellulose-based membranes.

The first three randomized prospective studies performed comparing bioincompatible versus biocompatible dialysis membranes demonstrated statistically significant decreases in mortality in patients dialyzed with biocompatible dialysis membranes (682–684). However, subsequent studies did not confirm these promising initial studies. In a prospective study of 57 patients with AKI, alternately assigned to a cuprophane bioincompatible versus polyamide bioincompatible membranes, the survival rate was no different—72% and 64%, respectively (685). In

another study of 133 consecutive ventilated patients with AKI, randomized to CRRT, with high-flux polyacrylonitrile versus polysulfone (low protein adsorption, low kinin generation), the mortality was 70% in both groups and there was no difference in renal recovery (686). In the largest trial to date on the subject, 180 patients with AKI were randomized to cuprophane bioincompatible versus polymethacrylate biocompatible dialysis membranes (687). High rates of hypotension were seen in both groups, and there was no difference in survival between groups: 42% with cuprophane and 40% with polymethacrylate. A prospective single-center study randomizing 159 patients with AKI to one of three dialyzer membranes—low-flux polysulfone, high-flux polysulfone, and a less biocompatible meltspun cellulose diacetate membrane—was recently reported (688). There was no significant difference between the three treatment groups for survival, time to renal recovery, and number of required dialysis treatments.

Conclusion

This chapter has reviewed the causes, pathophysiology, diagnosis, and management of AKI. Animal studies have greatly expanded our knowledge of the potential mediators of vascular and tubular cell injury in AKI. Several new clinical studies have been performed in humans. In Table 10-16 (689), the emerging new therapies for AKI are correlated with the pathophysiology.

Table 10–16 Emerging Therapies for AKI Correlated with the Pathophysiology (689)

Epithelial Cell Injury
Cysteine protease inhibitors (calpain or caspase inhibitors)
Selective NOS inhibition
Oxygen radical scavengers
NGAL
EPO
EPO receptor antagonist
Autophagy, mitophagy inducers
Inhibition of regulated necrosis (RIP1 or RIP3 inhibition)
Inhibition of microparticles
AMPK agonist

miR687, miR-24, miR-126 inhibition
miR-489 induction

Tubular Obstruction

Synthetic RGD peptides
Mannitol

Epithelial Repair

Growth factors
MSCs
Cell cycle

Leukocyte–Endothelial Interactions

Anti-ICAM, E-selectin antibodies
IL-18 binding protein
α-MSH
CRRT with increased ultrafiltration
Biocompatible dialysis membranes
Lymphocyte or macrophage depletion
Fractalkine receptor (CX3CR1) inhibition
Adenosine A_{1A}, A_{2A}, A_{2B} receptor agonists
TLR4 inhibition
NK or NKT cell depletion
Mast cell inhibition
HMGB1 inhibition
IL-33 inhibition
IL-17/IL-23 inhibition
IL-10 or Tregs
NF-κB inhibitors
Inflammasome inhibitors

Renal Vasodilatation

Atrial natriuretic peptide
Ca^{2+} channel blockers
Endothelin antagonists
Nitric oxide
MSCs

AKI to CKD transition

TGF-β inhibitor
Cell cycle inhibitors
JNK, ERK, PI3K, Akt inhibitors
Endothelin antagonists

AKI, acute kidney injury; NOS, nitric oxide synthase; NGAL, neutrophil gelatinase-associated lipocalin; EPO, erythropoietin; RGD, arginine–glycine–aspartic acid; CRRT, continuous renal replacement therapy; IL, interleukin; MSCs, Mesenchymal stem cells; Treg, regulatory T cell; NF-κB, nuclear factor-κB; CKD, chronic kidney disease; TGF-*β*, transforming growth factor-*β*; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase.

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CHAPTER 11

Chronic Kidney Disease: Manifestations and Pathogenesis

Michel Chonchol and Laurence Chan

Chronic kidney disease is characterized by a decrease in glomerular filtration rate (GFR) and histologic evidence of a reduction in nephron population. The clinical course is typically one of a progressive and unrelenting loss of nephron function, ultimately leading to end-stage renal disease (ESRD). However, the time between the initial onset of disease and ultimate development of ESRD may vary considerably, not only between different diseases but also in different patients with similar disease processes.

Assessment of Function in Chronic Kidney Disease

Assessment of GFR continues to be the most useful quantitative index of kidney function. Exogenous and endogenous markers have been used for the measurement of GFR. An ideal filtration marker should be freely filtered across the glomerular capillary wall and excreted only by glomerular filtration (1). Inulin fulfills all the criteria for an ideal filtration marker, and its renal clearance has been considered as a standard measure

of GFR. However, renal clearance of inulin requires precise regulation of an intravenous infusion of inulin to achieve a steady-state plasma inulin concentration and several timed urine collection with complete emptying of the bladder. Because of the inconvenience, it is only performed in research settings. Renal ^{125}I-iothalamate or ^{51}Cr-EDTA clearance after subcutaneous injection and timed urine collection also has been used as an alternative method (2,3).

van Slyke et al. introduced the concept of clearance in 1929 in their description of urea clearance. Blood urea nitrogen (BUN), however, is a less reliable indicator of kidney function, because factors other than the GFR—including protein intake, state of hydration, antianabolic agents (tetracycline and corticosteroids), blood in the bowel, fever, and infection —all can cause changes in BUN in the absence of changes in kidney function. In contrast, the blood level of creatinine, produced endogenously by the hydrolysis of phosphocreatine, provides a reasonable index of kidney function. Approximately 1 mg of creatinine is produced daily by the metabolism of 20 g of muscle (4). In addition, about 20% of urinary creatinine is derived from the ingestion of meat. Small quantities of creatinine are secreted by the renal tubules so that the creatinine clearance slightly overestimates true glomerular filtration. As a result, the 24-hour endogenous creatinine clearance generally exceeds inulin clearance, and this difference increases in patients with advanced chronic kidney disease and proteinuria. For clinical purposes, creatinine clearance is a simple and reliable method of estimating GFR and thus the degree of impairment of kidney function. Creatinine clearance (C_{cr}) can be estimated from serum creatinine (SrCr) determinations alone using the Cockcroft and Gault equation (5):

$$C_{cr}(\text{males}) = \frac{(140 - \text{age})(\text{weight [kg]})}{(72)(\text{SrCr [mg/dL]})}$$
$$C_{cr}(\text{females}) = \frac{(140 - \text{age})(\text{weight [kg]})}{(72)(\text{SrCr [mg/dL]})} \times 0.85 \qquad (11.1)$$

This equation corrects for the major factors that affect GFR, that is, age, sex, and weight. The normal creatinine clearance established by this method is 140 + 27 mL/minute for men and 112 ± 20 mL/minute for women.

A more accurate method to estimate GFR from serum creatinine was recommended by the Modification of Diet in Renal Diseases (MDRD)

study (6) which included 1,628 patients with diverse characteristics and causes of chronic kidney disease. The equation derived from this study, as shown in the following, predicts GFR by serum creatinine concentration (P_{cr}), demographic characteristics (age, gender, and ethnicity) as well as other serum measurements (serum urea nitrogen [SUN] and albumin [Alb]):

$$\begin{aligned}\text{GFR (mL/min/1.73 m}^2) = 170 \times (P_{cr}\ [\text{mg/dL}])^{-0.999} \times (\text{Age [y]})^{-0.176} \\ \times (0.762 \text{ if female}) \times (1.180 \text{ if African American}) \\ \times (\text{SUN [mg/dL]})^{-0.170} \times (\text{Alb [g/dL]})^{+0.318}\end{aligned} \quad (11.2)$$

This equation has less variability and is more accurate than other commonly used equations. Besides, it could be easily implemented and there is no need for 24-hour urine collections. It, however, is not very accurate with GFR values of 60 mL/minute or above. The MDRD study equation appears to be able to provide drug dosage adjustments similar to the Cockcroft and Gault equation. The new CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) (6) equation improves performance and risk prediction compared to the MDRD study equation in patients with a GFR of mL/minute or above. Current cystatin C-based equations (6) are not accurate in all populations, even in those with reduced muscle mass or chronic illness, where cystatin C would be expected to outperform creatinine. Estimated GFR, on the basis of prediction equations, reporting has led to a greater number of referrals to nephrologists, but the increased numbers do not appear to be excessive or burdensome.

The serum creatinine concentration doubles for every 50% reduction in GFR. For example, if a patient has a GFR of 100 mL/min/1.73 m^2 with a serum creatinine of 1 mg/dL, when the GFR falls to 50 mL/min/1.73 m^2 serum creatinine increases to 2 mg/dL. With a further fall in function to 25 mL/min/1.73 m^2, serum creatinine again doubles and is 4 mg/dL. As can be seen in Figure 11-1, changes in serum creatinine become a very sensitive method of estimating further impairment in kidney function when there is already extensive kidney damage. A plot of the reciprocal of the serum creatinine against time yields a straight line in many patients with chronic kidney disease. The linear decline in the reciprocal serum creatinine value with time is consistent with a linear loss of glomerular filtration. A change in the slope may indicate the superimposition of some additional factor that accelerates renal functional loss, such as volume depletion or a nephrotoxic agent if the slope is increased. Conversely, a

decrease in the slope represents slowing of the rate of decline in function. Irrespective of the underlying kidney disease, progression to ESRD is a common event once the serum creatinine exceeds 1.5 to 2.0 mg/dL. However, the rate of progression to ESRD occurs at a variable rate. When a patient is first seen with chronic kidney disease, it is most important to document the degree of renal impairment and attempt to determine if potentially reversible factors have contributed to the severity of kidney function decline.

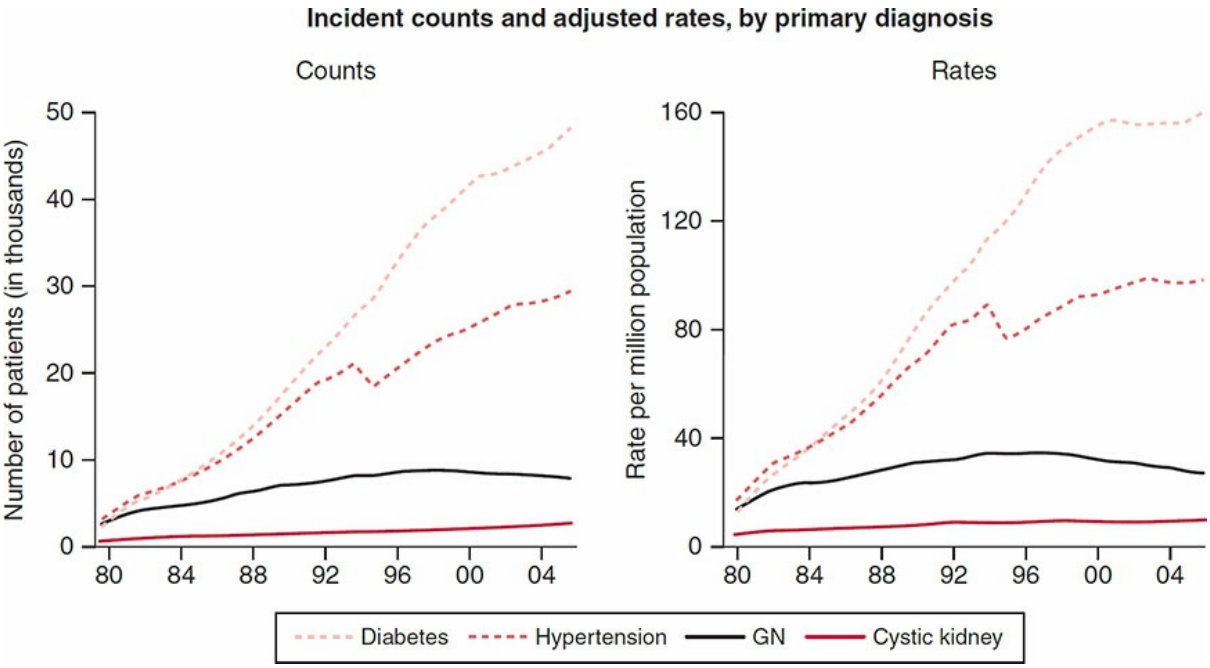

Figure 11–1 Incident counts and adjusted rates by major etiology for U.S. Medicare-treated end-stage renal disease.

Incidence and Prevalence of Chronic Kidney Disease

Chronic kidney disease is defined as the presence of kidney damage or GFR <60 mL/min/1.73 m^2 for 3 months or longer, irrespective of cause (6). Chronic kidney disease was divided into stages of severity (Table 11-1). The staging of chronic kidney disease is useful because it endorses a model in which primary physicians and specialists share responsibility for the care of patients with kidney disease. This classification also offers a common language for patients and the practitioners involved in the treatment of chronic kidney disease patients. For each stage of chronic kidney disease, the Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) provides recommendations for a

clinical action plan (Table 11-1) (7,8). Importantly, the staging system is based on estimated GFR and not only on the measurement of serum creatinine. The gradual decline of kidney function in patients with chronic kidney disease is initially asymptomatic. The earliest stages of chronic kidney disease are characterized by an apparent preservation of renal function of remaining nephrons. The basal GFR may be normal or even elevated because of hyperfiltration. Measurement of GFR after imposed stresses, such as after a high-protein meal, may reveal the absence of normal renal reserve. A diminution of renal reserve happens when GFR is reduced to 25% of normal. The patient usually has no symptoms, azotemia is present, and the serum creatinine is increased. As GFR falls to <25% of normal, an increasing number and severity of uremic clinical manifestations and biochemical abnormalities supervene. The magnitude of the population with chronic kidney disease is just beginning to be appreciated. Jones et al. (7) analyzed serum creatinine data from the Third National Health and Nutritional Survey (NHANES III), gathered between 1988 and 1994. Among men, the proportion of the population that had serum creatinine levels ≥1.5 mg/dL, ≥1.7 mg/dL, and ≥2.0 mg/dL were 4.98%, 1.87%, and 0.64%, respectively. Among women, the comparable figures were 1.55%, 0.73%, and 0.33%, respectively. On the basis of the population demography, the authors estimated that 6.2 million Americans have serum creatinine levels ≥1.5 mg/dL, 2.5 million have levels of ≥1.7 mg/dL, and 0.8 million have levels of ≥2.0 mg/dL. It is unclear as to what proportion of patients with abnormal serum creatinine progress to ESRD; however, there is increasing evidence that these patients develop irreversible but preventable complications of chronic kidney disease during this phase of renal insufficiency. More recently it was noticed that the prevalence of chronic kidney disease increased from 10.0% in 1988 to 1994 to 13.1% in 1999 to 2004, of the U.S. population. This increase was partly explained by the increasing prevalence of diabetes and hypertension (8).

Table 11–1 National Kidney Foundation Kidney Disease Outcomes Quality Initiative Classification, Prevalence, and Action Plan for Stages of Chronic Kidney Disease (7,8)

Stage	Description	GFR (mL/min/1.73	Estimated No of U.S. adults in	Action

			2000	
—	At increased risk	≥60 (with chronic kidney disease risk factors)	—	Screening: chronic kidney disease risk reduction
1	Kidney damage with normal or increased GFR	≥90	3,600,000	Diagnosis and treatment; treatment of comorbid conditions; slowing progression; CVD risk reduction
2	Kidney damage with slightly decreased GFR	60–89	6,500,000	Estimating progression
3	Moderately decreased GFR	30–59	15,500,000	Evaluating and treating complications
4	Severely decreased GFR	15–29	700,000	Preparation for kidney replacement therapy

GFR, glomerular filtration rate; CVD, cardiovascular disease.

The term “ESRD” is used for patients who are on renal replacement therapy (dialysis or transplantation) in order to avoid life-threatening uremia. The incidence of ESRD shows marked geographic variation as determined by the population base with regard to age, race, and sex. The reported incidence of ESRD in the United States in 2013 was 360 per million population (9). The prevalent rate of ESRD, adjusted for age, gender, and race, rose to reach 2,000 per million population in 2013. A similar increase in prevalence is occurring in most other industrialized countries as well; the reason for this increase in frequency of ESRD is unclear. In the United States, the distribution of patients reported by race most recently shows that 54.7% were white, 38.3% were African American, with the remaining 5.3% Asian/Pacific islanders and Native

American, with the remaining 5.3% Asian/Pacific islanders and Native Americans. Overall, 10.3% of the patients are Hispanic. In the U.S. population, it is clear that chronic kidney disease is more prevalent in the African American and Native American populations than in the white population.

Although life for chronic kidney disease patients can be sustained by chronic dialysis and kidney transplantation, neither form of therapy is totally satisfactory. The current yearly mortality rate in the U.S. dialysis population is over 20%. Results with renal transplantation have improved considerably with the advent of improved immunosuppressive therapy (9). The adjusted and averaged 1-year graft survival was over 90% for living related donors and ˜85% for cadaveric donor transplants (10,11). With improved transplant outcomes, growth in the number of patients wanting or needing a transplant has outpaced the supply of available organs. Although kidney transplant has become the preferred method of treatment for many ESRD patients, fewer than 20% of patients entering ESRD programs receive kidney transplantation because of age, associated disease, anatomic abnormalities of the urinary tract, the presence of preformed cytotoxic antibodies, or lack of availability of a suitable donor.

The rehabilitation rate of patients on chronic dialysis has been disappointing, and the cost of this treatment has been of increasing concern. Driven predominantly by recent growth in the ESRD patient population, total Medicare expenditures for the ESRD program alone have been increased steadily from $5 billion in 1991 to $30 billion in 2013 (9). Because of the cost as well as the morbidity and mortality associated with ESRD, every attempt should be made to preserve kidney function as long as possible, ideally preventing any further progression of the underlying renal disease.

Causes of Chronic Kidney Disease

The cause of kidney disease should be established, if possible, because some conditions may result in partial or full functional recovery if corrected. The major causes of chronic kidney disease found in patients entering the ESRD program are shown in Figure 11-2.

GLOMERULAR DISEASES

Diabetes mellitus has become the most common cause of chronic kidney

mellitus for more than 20 years will develop kidney disease. Although the incidence of ESRD in patients with type 2 diabetes may be less than that found in type 1 diabetes, because of the larger number of patients with type 2 diabetes it is a more frequent cause of ESRD than type 1 diabetes (36.5% vs. 7.2%) (11). Of note, in the United States at least 80% of diabetic patients with ESRD are type 2 diabetic (9).

Glomerulonephritis represents the third most common cause of ESRD. The most common glomerular diseases are focal segmental glomerulosclerosis (FSGS) and membranoproliferative and lupus glomerulonephritis. However, it should be noted that the majority of glomerular diseases are unclassified. It is possible that this disease accounts for a relatively large fraction of unclassified glomerular diseases because immunoglobulin A (IgA) nephropathy is the most common glomerular disease responsible for ESRD in most other developed countries.

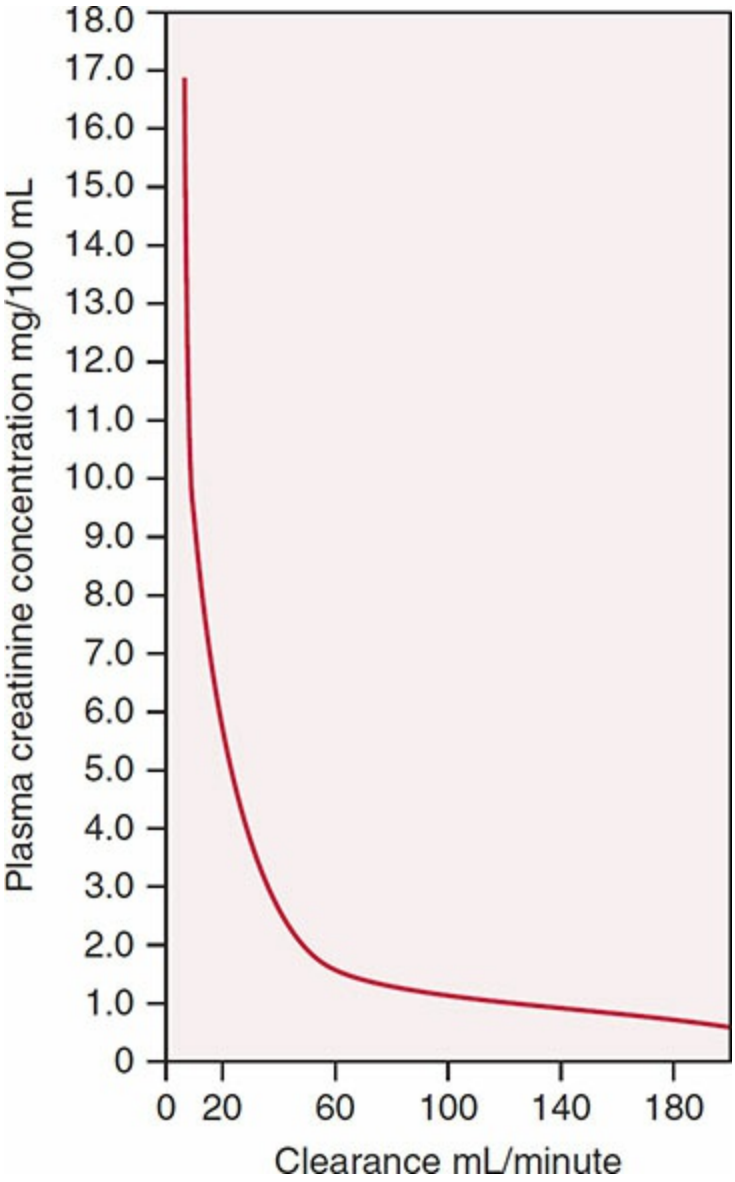

Figure 11–2 Relationship between serum creatinine concentration and creatinine clearance. (Reprinted from Doolan PD, Alpen EL, Thiel GB. A clinical appraisal of the plasma concentration and endogenous clearance of creatinine. *Am J Med.* 1962;32(1):65–79, with permission from Elsevier.)

VASCULAR DISEASE

Hypertension is the second leading reported cause of ESRD. A 15-year follow-up study of 361,659 men with hypertension found that 924 developed ESRD (12). This represented an incidence of 17.12 per 100,000 person-years. The relative risk for development of ESRD for diastolic blood pressure >120 mm Hg versus <70 mm Hg was 30.9. For systolic blood pressure >200 mm Hg versus <120 mm Hg, the relative risk was 48.2. Across the entire range, blood pressure represented an independent risk factor for kidney disease progression. The relative risk for African

Americans was 1.99 (12). This increased risk could not be explained by difference in levels of systolic or diastolic pressures or other known risk factors. In general, ESRD secondary to hypertension occurs in African American patients with a long history of uncontrolled hypertension or almost any patient with a history of malignant or accelerated hypertension (13–15). Although the incidence of chronic kidney disease from hypertension can be markedly attenuated by treatment of accelerated or malignant hypertension (13,16), adequate chronic treatment of milder hypertensive states, especially in the African American population, may not prevent progression of kidney disease (12,14).

Other less common vascular causes of chronic kidney disease are atheroembolic disease and bilateral renal artery stenosis. Atheroembolic disease should be suspected in any individual who develops progressive decrease in kidney function following a vascular diagnostic procedure or surgery. In contrast to other vascular renal disease, atheroembolic disease may include high-grade proteinuria, eosinophiluria, and decreased serum complement. Diagnosis of atheroembolic disease largely depends on renal biopsy in which the cholesterol clefts are observed. There is no specific treatment for atheroembolic disease. Bilateral renal artery stenosis, as a cause of ischemic nephropathy, is suggested by a further reversible reduction in renal function precipitated by converting enzyme inhibitors.

Arteriography usually is required for the diagnosis of renal artery stenosis. As of this time, there is no consistent evidence that kidney function can predictably be improved in patients with bilateral renal artery stenosis by either angioplasty or surgical correction of the lesions. Uncontrolled studies, however, have suggested that these procedures can improve kidney function in some instances.

INTERSTITIAL NEPHRITIS

Interstitial nephritis is a descriptive term implying fibrosis and an inflammatory response in the interstitium of the kidney. The glomeruli are involved only secondarily as a result of the fibrosis and vascular changes. Because of the potential reversibility or prevention of this group of renal diseases, it is important to differentiate interstitial nephritis from glomerulonephritis.

A number of clinical and biochemical features, listed in Table 11-2, tend to separate these two forms of renal disease. Characteristically, patients with interstitial nephritis complain of polyuria and nocturia. Their urine volume is unusually large (3 to 5 L/day) because the kidney’s ability

to concentrate urine is lost early in the course of kidney disease. The diluting capability in interstitial nephritis is maintained even late in the course of kidney disease; thus, the urine osmolality and specific gravity may be low when determined on a random collection of urine.

A feature of advanced glomerular diseases is high-grade proteinuria, which usually is in excess of 2.5 g/day. Even with advanced interstitial nephritis, the 24-hour urinary protein excretion is usually <1 to 2 g. Furthermore, the urinary protein may be predominantly an α_2- or β-globulin instead of albumin. In interstitial nephritis, serum uric acid is commonly elevated, and in one type of interstitial nephritis—lead nephropathy—clinical gout has been recognized in ˜50% of the patients (17,18). The urinary sediment in interstitial nephritis may be totally unremarkable, or there may be a few white blood cells (WBCs) and hyaline casts. Renal salt wasting appears to be more common in patients with interstitial nephritis than in other forms of kidney disease, and salt supplementation sometimes must be given to maintain extracellular fluid (ECF) volume. Finally, hypertension is less common in interstitial nephritis and anemia may be disproportionately more severe for the degree of compromised kidney function than in chronic glomerulonephritis.

As is apparent in Table 11-3, a variety of drugs and toxins can be the etiologic agent responsible for causing interstitial nephritis. In general, with the exception of analgesics, drugs cause an acute interstitial nephritis that is reversible when the drugs are discontinued. The severity and chronicity of other forms of interstitial nephritis are largely related to the amount and duration of exposure to the various nephrotoxins. Interstitial nephritis accounts for 3% of the patients in this country being treated for ESRD. In this group, currently analgesic nephropathy accounts for 0.8% of patients being treated for ESRD. Analgesic nephropathy used to account for up to 20% of ESRD in several countries (19). However, following the removal from the market of analgesics containing the combination of aspirin and phenacetin, the incidence of this disease has markedly decreased worldwide. The typical patient with this disease is a depressed, middle-aged woman who gives a history of years of daily ingestion of analgesics containing caffeine, aspirin, and phenacetin. Usually, the total consumption of analgesics amounts to several kilograms. Patients frequently complain of headaches, backache, or other types of chronic pain and state that the analgesics are consumed to relieve this pain. There is evidence that sometimes the headaches may result from the caffeine or phenacetin ingestion, or both. The headaches may disappear if the patient can be persuaded to discontinue the analgesics. The patient commonly

presents with recurrent urinary tract infections, gross hematuria, or symptoms of uremia. However, because papillary necrosis is common, acute kidney injury, and ureteral colic may develop as a result of the passing of necrotic papillae down one or both ureters. In this disease, the kidney has a remarkable capacity to recover even after what would appear to be a terminal state of ESRD (20). With conservative treatment and discontinuation of the analgesics, the patient can achieve significant improvement in kidney function and have a relatively good, long-term survival. Many times, however, it is very difficult to convince the patient to break a long habit of drug abuse.

Table 11–2 Features Differentiating Glomerulonephritis and Interstitial Nephritis

Feature	Glomerulonephritis	Interstitial Nephritis
Proteinuria	>3 g	<1.5 g
Sediment	Numerous cells and red blood cell casts	Few cells and casts
Sodium handling	Normal until late	Sodium wasting
Anemia	Moderate severity until late	Disproportionately severe for degree of renal failure
Hypertension	Common	Less common
Acidosis	Normochloremic	Hyperchloremic
Uric acid	Slightly elevated	Markedly elevated
Urine volume	Normal	Increased

Table 11–3 Various Etiologies of Interstitial Nephritis

Analgesics
Other Drugs
Sulfonamide Penicillin and homologs Furosemide, thiazides

Phenindione
Phenytoin
Cimetidine
Nonsteroidal antiinflammatory drugs
Calcium Disorders
Hyperparathyroidism
Milk–alkali syndrome
Sarcoid
Neoplasms
Multiple myeloma
Uric Acid
Gouty nephropathy
Hematologic disorders
Oxalate Deposition
Associated with small bowel disease
Hereditary
Anesthetic agents: methoxyflurane
Ethylene glycol
Heavy Metals
Lead
Cadmium
Uranium
Copper
Miscellaneous
Infection
Idiopathic

Uric acid and oxalate nephropathy and cystinosis represent <0.1% each of the ESRD population (11). Chronic kidney disease is uncommon in patients with primary gout, and when it does occur, it is slowly progressive and only becomes clinically important late in life (20). However, in some hematologic disorders, particularly in association with the use of chemotherapeutic agents, there may be marked overproduction of uric acid, which may cause acute kidney injury caused by deposition of urate crystals in the tubules.

Another compound capable of inducing a severe interstitial nephritis is oxalate. Besides ethylene glycol intoxication (21), increased urinary excretion of oxalate can occur in association with genetic disorders as well as with a number of acquired conditions. Two enzymatic defects have been described that can result in the accumulation of glyoxylic acid and hyperoxaluria. In the first type, urinary excretion of oxalic acid, glyoxylic acid, and glycolic acid is increased as a result of deficiency of 2-oxoglutarate-glyoxylate carboligase (22). In the second defect, urinary excretion of glycolic acid is normal, but the excretion of L-glyceric acid and oxalate is increased. This condition owes to a deficiency of D-glyceric dehydrogenase (22). Both diseases are characterized by nephrolithiasis, nephrocalcinosis, and ESRD, with few patients living beyond the age of 40 years.

Recently, a number of acquired forms of hyperoxaluria and kidney disease have been described. Methoxyflurane anesthesia can cause hyperoxaluria and azotemia (23). In addition, it has been recognized that patients with distal small bowel disease may have hyperoxaluria (24). In this group of patients, calcium oxalate stones are common; however, marked oxalate deposition occasionally may occur in the kidney, resulting in interstitial nephritis and loss of kidney function. The mechanism responsible for hyperoxaluria has been shown to be a consequence of increased absorption of dietary oxalate (24). It is felt that this results from calcium and possibly magnesium (which normally binds oxalate in the gut, rendering it insoluble and nonabsorbable) being bound to fatty acids in steatorrheic states, allowing the oxalate to be absorbed. Similarly, this condition has been treated successfully by giving supplemental calcium. Furthermore, cholestyramine also has been shown to be effective in decreasing oxalate absorption from the bowel and thus in decreasing urinary excretion of this compound (24).

All other causes of interstitial nephritis are even less prevalent. Conditions that cause hypercalcemia, hypercalciuria, or both can lead to the deposition of calcium in the kidney, with a resulting interstitial nephritis. Radiographic evidence of nephrocalcinosis is frequently a late finding and even then may be observed only by using the technique of nephrotomography. Thus, radiographic evidence of nephrocalcinosis cannot be relied on to establish the diagnosis even when kidney function is severely impaired. In this condition, if the underlying cause responsible for the disturbance of calcium metabolism such as primary hyperparathyroidism, sarcoid, or milk–alkali syndrome is corrected or treated, further progression of kidney disease can be either slowed or

prevented (25,26).

A final group of agents that can produce a chronic interstitial nephritis are some of the heavy metals, including copper, lead, cadmium, and uranium. Lead nephropathy is common in Queensland, Australia (18), and has been reported in some areas of the United States in patients who have consumed moonshine whiskey (27). Lead nephropathy may occur more commonly than previously suspected in this country. Batuman et al. (28) have suggested that patients having the combination of interstitial nephritis and gout should be suspected of having lead nephropathy. This supposition was supported by the finding that ethylenediaminetetraacetic acid (EDTA) mobilized significantly greater amounts of lead in patients with kidney disease and gout than in patients with either gout or chronic kidney disease alone. Cadmium intoxication also can lead to an interstitial nephritis and renal tubular dysfunction. Characteristically, patients present with aminoaciduria, glycosuria, phosphaturia, and severe osteomalacia (29). As a result of industrial contamination with the element, chronic cadmium intoxication is especially prevalent in the people living along the Jinzū River in Japan (29). In Wilson disease, copper is deposited in the proximal tubule cells and may cause a variety of renal functional abnormalities, including Fanconi syndrome, proteinuria, and hematuria; however, it does not appear to progress to ESRD.

Evidence suggests that, in the adult, chronic urinary tract infection without obstruction rarely, if ever, leads to ESRD. However, there are some exceptions in which renal bacterial infections can lead to chronic kidney disease if untreated; among them are tuberculosis, multiple renal abscesses, and bacterial infections associated with papillary necrosis.

Because a number of patients with interstitial nephritis have a potentially preventable or reversible form of kidney disease, a careful history should be obtained relating to medications, small bowel disease, and possible environmental exposure to some toxin. In addition, serum and urinary uric acid and calcium should be determined. In selected cases, urinary oxalate excretion should be measured and heavy metal screens performed. The normal values to be used for these screening procedures are given in Table 11-4.

REFLUX NEPHROPATHY

Reflux nephropathy is the second most common kidney disease in children (30). According to the European Dialysis and Transplantation Association, it accounts for 30% of advanced kidney disease in children <16 years. The

infant kidney is especially susceptible to intrarenal reflux. Most evidence would suggest that scarring usually occurs by 2 years of age (31) and that new scarring is unusual after age 5 (31–33). Increasing evidence suggests that severe congenital kidney damage already may be present at birth (31). This may represent a disorder in kidney embryogenesis as a result of an abnormal development of the ureteral bud. Recent evidence also suggests that this condition may have a heritable basis with contribution from several genetic foci. Prognosis is largely determined by the extent to which the kidney is scarred and contracted when the patient is initially seen. It has been shown also that the severity of the reflux can be correlated with the degree of kidney damage and that surgical correction of reflux is associated with eradication of upper urinary tract infection and improvement in renal growth and function. However, a recent study in children suggests that surgical correction of reflux offers no advantage over good medical management (33). Although there are no control trials in adults regarding surgical correction of reflux, most studies suggest that it does not influence the course of kidney disease.

Table 11–4 Normal Values Used in Screening Patients with Interstitial Nephritis

Substance Measured	Plasma	Urine
Calcium	9.5–10.5 mg/dL	<300 mg/d
Oxalate	30 ng/dL	<40 mg/d
Uric acid	5–7 mg/dL	<800 mg/d
Lead	<40 ng/dL	<0.5 mg/d[a]
Cadmium, mercury, and uranium	Normally nondetectable	Normally nondetectable

[a]Following 1 g of ethylenediaminetetraacetic acid.

HEREDITARY RENAL DISEASE

Approximately 5% to 8% of patients with chronic kidney disease have an hereditary etiology such as autosomal dominant polycystic kidney disease (ADPKD), Alport syndrome, Fabry disease, congenital nephrotic syndrome, medullary cystic disease, cystinosis, or familial amyloidosis. This is another group of kidney diseases for which specific treatment is not

available (34). Through genetic counseling, however, a number of these diseases are potentially preventable. Therefore, the physician has an obligation to advise potential parents of the risk of having children with kidney disease and to determine when possible which family members are at risk or have diagnosable kidney disease. In ADPKD, which is inherited as an autosomal dominant disorder with complete penetrance, a DNA probe has localized the majority of cases (>90%) to a mutation in the short arm of human chromosome 16 (PKD1). This technique has been used to diagnose the disease in utero in a 9-week fetus (35). A mutation of chromosome 4 (PKD2) accounts for ˜10% of patients with the disease. The genes for PKD1 and PKD2 have been identified. PKD1 encodes polycystin. An abnormality in polycystin may impair cell–cell and cell–matrix interactions, leading to abnormal epithelial cell differentiation and various phenotypic expressions (36,37). The PKD2 gene encodes for a channel protein. Mutation of the same leads to decrease cellular calcium with increased cyclic AMP that has been known to contribute to cyst formation.

The potential success of genetic counseling for hereditary diseases is demonstrated by a study carried out at the genetic clinic at the Hospital for Sick Children in London. Approximately two-thirds of the families who were informed that the chances were >10% that their children would develop hereditary disease decided to have no more children, whereas three-fourths of families informed that the chances were ≤10% elected to have more children (36).

Risk Factors for Development of End-Stage Renal Disease

The four important risk factors for the development of ESRD are race, age, sex, and family history.

RACE AND ETHNICITY

Male African Americans aged 25 to 44 years are 20 times more likely to develop kidney disease secondary to hypertension than white men (15,38). African Americans also have a very high incidence of idiopathic FSGS as well as that associated with intravenous drug use and acquired immunodeficiency syndrome (AIDS) (9,39). The attack rate of FSGS in

African American men with AIDS is ˜10 times as great as in white men. In fact, FSGS is the most common cause of kidney disease in young adult African American men. African Americans also have a fourfold greater risk than whites of developing ESRD from type 2 diabetes (11). In contrast, two diseases, ADPKD and especially IgA nephropathy, occur with considerably less frequency in the African American than in the white population. In Native Americans, diabetes accounts for almost twice as much ESRD (68.2%) as found in the white or African American population. Hispanics also have a high frequency of diabetic ESRD, with some reports as high as 60% (9).

AGE

Since 2000, the adjusted incident rate of ESRD has increased by 11.0% for patients ≥75 years, while the rate for those age 20 to 44 years has grown by 6.1% (9). The incidence of diabetic kidney disease also increases dramatically with age. However, in contrast to the total causes of chronic kidney disease, which continue to increase with advanced age, over 66% of diabetic ESRD occurs before 64 years of age. Before age 40, FSGS, lupus erythematosus, Henoch–Schönlein purpura, AIDS-related nephropathy, and congenital and hereditary disease (e.g., renal agenesis, obstructive nephropathy, Alport syndrome, and reflux nephropathy) are most commonly seen. In the age group 40 to 55 years, ADPKD, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and hemolytic uremic syndrome are seen with increasing frequency. Goodpasture syndrome, interstitial nephritis, analgesic nephropathy, amyloidosis, multiple myeloma, and Wegener granulomatosis are the most common diseases in the age group >55 years.

SEX

Sex is an additional risk factor for the development and progression of certain types of kidney disease. Overall, the incidence of ESRD is greater in males than in females (9). However, there are certain causes of ESRD that occur more frequently in females, such as lupus erythematosus, scleroderma, and hemolytic uremic syndrome/thrombotic thrombocytopenia purpura.

FAMILY HISTORY

Genetic factors also are important in predisposing individuals to developing ESRD. Patients with diabetes who have a family history of essential hypertension and abnormal lithium–sodium countertransport are at greater risk of developing chronic kidney disease (40,41). Both candidate locus and genome-wide strategies have been used to target genes that contribute to the risks for development of these orders. Similarly, there are numerous types of hereditary renal disease such as Alport syndrome and ADPKD, plus a variety of less common and largely recessive or sex-linked hereditary diseases such as Fabry disease, tuberous sclerosis, medullary cystic disease, sickle cell disease, familial Mediterranean fever, type 1 glycogen storage disease, cystinosis, oxalate nephropathy, and infantile PKD (42–44).

Individual variability in the rate of progression to ESRD is a characteristic feature among patients with either inherited or acquired causes of kidney diseases. A number of genetic foci that contribute to the progression of chronic kidney disease have been identified. Most extensively studied has been an insertion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene. Studies in a variety of disorders have revealed an important contribution of this locus in the progressive deterioration of kidney function. The two different alleles defined by this polymorphism of the ACE gene are associated with corresponding differences in the endogenous activity of the encoded enzyme. The homozygous deletion/deletion (D/D) variant is associated with the highest expression of endogenous activity and a greater risk of progression to ESRD. This group of patients with the DD polymorphism of the ACE gene may also be more likely to have an antiproteinuric response to ACE inhibitors (45–47).

Symptomatology of Chronic Uremia

Early in chronic kidney disease, when the GFR is >25 mL/min/1.73 m^2 (i.e., ˜25% of normal), the majority of patients have few symptoms, and the biochemical abnormalities are equally unremarkable. Although a rise in serum uric acid has been reported to occur early in kidney disease, the increment usually is <1 mg/dL (18). Therefore, with the exception of some patients with interstitial nephritis, secondary gout is uncommon in ESRD. Proteinuria is common at this stage and the nephrotic syndrome may be present in some glomerular diseases. In association with high-grade

proteinuria, the patient may also lose antithrombin III, with resulting antithrombin III deficiency, a hypercoagulable state, and a predisposition to thromboembolic complications (48). The third major finding in the early stage of kidney disease is hypertension. If the hypertension is not treated, arteriolar nephrosclerosis as well as focal glomerulosclerosis may develop and accelerate the loss of kidney function. Because it is extremely difficult to determine whether the progressive loss of kidney function is a consequence of the underlying kidney disease or the hypertensive state, it is imperative that blood pressure be well controlled.

FLUID AND ELECTROLYTE DISTURBANCES

Disturbances of fluid and electrolytes may occur as the GFR falls below 25 mL/min/1.73 m^2. The interesting aspect is that on a normal diet, even with a GFR of 3 to 5 mL/min/1.73 m^2, there may be only minimal disturbances of plasma electrolytes and the body water content. This is a result of the fact that as GFR falls there is increased fractional clearance of electrolytes, as well as water. This has been termed “the magnification phenomenon” by Bricker et al. (49). This implies that the diseased kidney continues to be under the control of a variety of biologic systems that regulate the excretion of the various electrolytes, and the excretory response per nephron evoked by these systems varies inversely with the number of surviving nephrons. Because of this, the individual with advanced chronic kidney disease is able to excrete the elements and waste products obtained from a normal dietary intake, maintaining reasonable water and electrolyte balance.

However, the range over which the individual can maintain balance is limited with advanced chronic kidney disease. Because of the impaired capacity to dilute or concentrate urine, the patient will develop increasing dehydration and hypernatremia if water intake is restricted; and the degree of azotemia may increase secondary to further impaired excretion of nitrogenous waste products. Conversely, hyponatremia may occur if water intake is excessive.

When placed on a low-sodium diet, the majority of patients with advanced chronic kidney disease are unable to reduce urinary sodium excretion to the level of their sodium intake, or it takes three to four times longer to do so than in a normal person. In a few patients, usually with medullary cystic disease, ADPKD, or interstitial nephritis, an excess sodium intake may be necessary to maintain sodium balance (50). A rare patient may require as much as 10 to 20 g of salt supplementation daily to

maintain ECF volume and maximum kidney function. In general, such severe renal salt wasting is very infrequent and occurs in the presence of far-advanced kidney disease.

Hyperkalemia rarely occurs in patients who have a GFR >25 mL/min/1.73 m^2 in the absence of an endogenous or exogenous potassium load. Potassium balance is maintained in the majority of patients by a combination of increased tubular secretion of potassium, which is mediated in part by aldosterone (51,52) and the increased fecal potassium loss (51,52). Because these mechanisms must work to the maximum in advanced kidney disease, there are several circumstances in which hyperkalemia may develop. Competitive inhibition of aldosterone with spironolactone, or inhibitors of distal potassium secretion (e.g., amiloride or triamterene) may induce severe hyperkalemia. A second cause of hyperkalemia is an increased intake of potassium; and third is acute acidosis that caused intracellular potassium to be released into the extracellular pool. A rough clinical estimate of the effect of acidosis on serum potassium concentration is as follows: for every decrease of 0.1 pH unit, serum potassium increases by ˜0.6 mEq/L. β-Blockers, nonsteroidal antiinflammatory drugs (NSAIDs), ACE inhibitors, and angiotensin receptor blockers (ARBs) also may interfere with the renin–angiotensin system and lead to hyperkalemia.

Schambelan et al. (53) described an additional cause for the spontaneous occurrence of hyperkalemia in patients with kidney disease. Although all their patients had hyperkalemia in association with chronic kidney disease, the degree of kidney function impairment often was not severe. The majority of their patients had either diabetes mellitus or interstitial nephritis (53). The highlight of the findings in these patients was diminished plasma levels of renin and aldosterone. Studies suggest that the hyperkalemia is a result of hypoaldosteronism, which is attributable to hyporeninemia. The diminished plasma renin activity may in turn result from an autonomic neuropathy or sclerosis of the juxtaglomerular apparatus in the diabetic patients. Sickle cell disease, kidney transplantation, and lupus nephritis also have been associated with hyperkalemia, probably secondary to diminished tubular secretory capacity. Another cause of hyperkalemia occurs in some patients with chronic obstructive uropathy (54). These individuals appear to have a tubular resistance to aldosterone in contrast to the hyporeninemic–hypoaldosterone patients. Thus, these conditions should be considered when hyperkalemia is noted in patients with chronic kidney disease and other causes have been excluded.

Hypokalemia also may occur in patients with chronic kidney disease. A number of factors may be responsible for this finding, including poor dietary intake of potassium, diarrhea, diuretic therapy, metabolic alkalosis with secondary hyperaldosteronism, or specific renal tubular defects such as those found in association with type 1 renal tubular acidosis (RTA) and Fanconi syndrome (type 2 RTA).

Total body burdens of other elements, although not totally corrected by the diseased kidney, are corrected to the extent that the remaining alterations are associated with few, if any, clinical symptomatology until ESRD has occurred. The fractional clearance of phosphorus, magnesium, and calcium all increase as GFR progressively falls. As a result, plasma magnesium and phosphorus are not elevated until GFR falls below 25 mL/min/1.73 m^2 (55). Even then, plasma values rarely increase >1 to 2 mg/dL until GFR falls below 5 mL/min/1.73 m^2. The serum magnesium concentration may be slightly elevated when the patient is ingesting a normal magnesium intake. Magnesium-containing antacids and laxatives should be avoided because such patients have difficulty in excreting large magnesium loads (56).

Although fractional clearance of calcium is increased in kidney disease, absolute excretion is actually decreased. In contrast to other elemental disturbances, there may be major consequences as a result of the altered calcium metabolism associated with the uremic state. Parathyroid hormone (PTH) levels are found to significantly increase when GFR falls to 45% of normal, and 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) levels fall when GFR is 70% to 80% of normal. Hypocalcemia also is a common finding in patients with advanced kidney disease. Hypocalcemia probably results from a combination of factors including low $1,25(OH)_2D_3$ with decreased gastrointestinal absorption of calcium, hyperphosphatemia, and bone resistance to the calcemic effect of PTH.

ACID–BASE DISORDERS

Acidosis is a common disturbance at a more advanced stage of chronic kidney disease. Normally, the kidneys are responsible for excreting 60 to 70 mEq of hydrogen ions daily. Although the urine can be acidified normally in a majority of patients with chronic kidney disease (57), these patients have a reduced ability to produce ammonia. With advanced kidney disease, total daily acid excretion is usually reduced to 30 to 40 mEq; thus, throughout the remainder of their course of chronic kidney

disease, many patients may be in a positive hydrogen ion balance of 20 to 40 mEq/day. The retained hydrogen ions probably are buffered by bone salts, although this has not yet been unequivocally proven. On occasion, hyperchloremic RTA with a normal anion gap may occur in the early stage of kidney disease. With more advanced chronic kidney disease, the plasma chloride concentration becomes normal, and a fairly large anion gap may develop. In most patients with chronic kidney disease, the metabolic acidosis is mild, and the pH rarely is <7.35. As with other abnormalities in chronic kidney disease, primary symptomatic manifestations of acid–base disturbances occur when the patient receives an excessive endogenous or exogenous acid load or loses excessive alkali (e.g., diarrhea).

The final stage of chronic kidney disease occurs when the GFR falls below 10 mL/min/1.73 m^2. The deranged metabolic functions present at this stage of kidney disease are responsible for the striking clinical features of uremia.

ANEMIA

The prevalence of anemia increases with progression of kidney disease and 90% of patients with a GFR <25 mL/min/1.73 m^2 have anemia defined as a hemoglobin <12 g/dL (58). The anemia of chronic kidney disease has been felt to result from a combination of factors, including reduced erythropoietin (EPO) activity, circulating factors that appear to inhibit the bone marrow response to EPO, and shortened erythrocyte life span. Red blood cell (RBC) survival is decreased from 120 to 80 days in chronic kidney disease patients. Both metabolic and mechanical factors contribute to this short life span of RBC. Almost all patients with chronic kidney disease have much lower baseline EPO levels than those of normal subjects at the same degrees of anemia. Patients with ADPKD are the exception and usually have higher EPO levels with less severe anemia. EPO, a glycosylated, 165 amino acid protein produced by renal peritubular capillary endothelial cells, acts on erythroid progenitor cells in the bone marrow. With the recent availability of recombinant EPO, it appears that the major cause of anemia has been a failure of EPO production by the diseased kidney, because uremic patients typically respond so well to exogenously administered EPO (58).

Low hemoglobin levels have been associated with increased left ventricular hypertrophy and cardiovascular outcomes in patients with kidney disease. Therefore, several studies have evaluated whether treatment of anemia results in improved outcomes in chronic kidney

disease patients. The United States Normal Hematocrit Trial (59) of chronic hemodialysis patients with cardiac disease randomly assigned patients to a target hematocrit of 42% or 30%. The primary outcome was time to death or nonfatal myocardial infarction (MI). The study was stopped early as the group assigned to the higher hematocrit had an increased risk of mortality that was trending toward statistical significance and a higher rate of adverse vascular access events due to thrombosis. In the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) (60) trial, 1,432 patients with moderate and advanced chronic kidney disease and a hemoglobin <11 g/dL were randomized to achieve a target hemoglobin of either 11.3 or 13.5 g/dL. The CHOIR trial was also terminated early as a significantly higher number of cardiovascular events was observed in the higher hemoglobin group. Of note, patients in the higher hemoglobin group did not reach the target of 13.5 g/dL; they only reached a mean hemoglobin concentration of 12.6 g/dL and despite randomization, the higher hemoglobin group had more cardiac comorbidities, which could have contributed to the adverse outcomes observed in these patients. Similarly, the Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE) (60) trial randomly assigned 603 patients with moderate and advanced chronic kidney disease and anemia to achieve a target hemoglobin of normal (13–15 g/dL) or low normal (10.5–11.5 g/dL). After 3 years of follow-up, both groups had a similar risk of achieving the primary end point (composite of cardiovascular events) and the higher hemoglobin group had increased quality of life and general health. Treatment of anemia did not have any effect on left ventricular hypertrophy, as the left ventricular mass index remained unchanged in both groups.

The optimal hemoglobin level remains controversial and the current K/DOQI recommendations are not to exceed a hemoglobin level of 12 g/dL in chronic kidney disease patients (60). The Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) (60), which enrolled more patients than the CHOIR and CREATE trials combined, demonstrated similar results. The use of darbepoetin alfa in patients with diabetes, chronic kidney disease, and moderate anemia who were not undergoing dialysis did not reduce the risk of either of the two primary composite outcomes (either death or a cardiovascular event or death or a renal event) and was associated with an increased risk of stroke (60).

BLEEDING DIATHESIS

Disturbances in the coagulation system also occur with an advanced stage of chronic kidney disease. Approximately 20% of uremic patients have a modest degree of thrombocytopenia, but it is rare to find a platelet count of <50,000. Severe thrombocytopenia may occur in patients with the hemolytic uremic syndrome as a consequence of disseminated intravascular coagulation. However, this is not a common cause of chronic kidney disease in adults. Platelet factor 3 is reduced and platelet aggregation is decreased (61) in advanced kidney disease. This results in prolongation of the Ivy bleeding time and poor clot retraction. However, platelet function is caused by multiple factors such as the retention of uremic toxins, nitric oxide, anemia, and hyperparathyroidism. The importance of uremic toxins is suggested by the beneficial effect of acute dialysis on platelet dysfunction. How uremic toxins might interfere with platelet function is not completely understood. In vitro studies suggest that a dialyzable factor might interfere with the binding of fibrinogen. Uremia-induced changes in nitric oxide production also may contribute to inhibition of platelet aggregation. Treatment of the uremic state with dialysis improves platelet function in the majority of patients, suggesting some dialyzable factor is responsible for this abnormality. It is of interest that D-deaminoarginine vasopressin (dDAVP) improves bleeding time without affecting platelet abnormalities (62). This suggests that an abnormality in factor VIII or von Willebrand factor may play a role in the pathogenesis of the bleeding abnormality in the uremic state (62). Anemia also contributes to the abnormal bleeding time present in uremic patients. A higher hematocrit causes the platelets to skim at the endothelial surface, which is optimal for platelet–endothelium interaction. Such skimming does not occur with hematocrits <25% to 30%.

SEROSITIS

Another complication noted with some frequency in patients with far-advanced chronic kidney disease is involvement of the serous membranes as manifested by pericarditis and pleuritis. The involved membrane is markedly thickened, extremely vascular, and infiltrated with plasma cells and histiocytes (63). Both pleural and pericardial friction rubs may be heard. When pleural and pericardial effusions are present, they are uniformly hemorrhagic and usually contain fewer than 10,000 WBCs/mm^2. Pericardiocentesis, as well as thoracentesis, is occasionally necessary to relieve clinical symptoms. However, if the uremic state is not improved with treatment of reversible factors or hemodialysis, recurrent

effusions are common. Rarely, constrictive pericarditis may follow healing of acute uremic pericarditis.

Chronic ascites also may be a manifestation of uremic serositis and advanced kidney disease. This complication arises primarily in patients who have had previous abdominal surgery or peritoneal dialysis. The ascitic fluid is an exudate with the ascitic fluid albumin/plasma albumin concentration ratio of >0.5. Although fluid overload may worsen uremic ascites, fluid removal frequently is not a successful mode of treatment. Renal transplantation or several consecutive days of intensive dialysis has been useful in treating uremic ascites.

GASTROINTESTINAL DISORDERS

Most patients with far-advanced kidney disease have gastrointestinal symptoms that are a major part of their clinical picture (64). Specifically, nausea, vomiting, and anorexia are extremely common. Uremic stomatitis, characterized by dry mucous membranes and multiple, bright-red, small, ulcerative lesions, may occur with advanced uremia. Poor dental hygiene appears to contribute to the development of uremic stomatitis. Because saliva in uremic patients has an increased urea content, it has been suggested that the stomatitis results from high levels of ammonium, which is formed by bacteria ureases from the high levels of salivary urea. Inflammation of salivary glands (e.g., parotitis) also may occur in uremic patients and is usually associated with stomatitis. The salivary glands may become markedly swollen in chronic kidney disease, but characteristically they are not tender or indurated, as might be seen in inflammatory parotitis.

Pancreatic involvement also has been found on postmortem examination in patients who have died from uremia. Typically, on histologic examination of the pancreas, there is dilatation of the acini, flattening of the epithelial cells, and inspissation of the intraacinar secretions. Clinical symptoms of pancreatitis also may occur. It was felt previously that uremia alone, as a result of chronic loss of kidney function and thus the inability to excrete amylase, could significantly elevate the serum amylase concentration, but this has been shown not to be the case (65). Rather, when a high elevation of serum amylase concentration is found in patients with chronic kidney disease, pancreatitis should be considered strongly. In acute kidney injury, however, serum amylase elevations are common but are rarely over twice normal in the absence of clinical evidence of pancreatitis (65). Other findings in the gastrointestinal

tract in advanced uremia include erosive gastritis and uremic colitis characterized by submucosal hemorrhages and small mucosal ulcerations. With the exception of anorexia, nausea, and vomiting, the majority of other gastrointestinal complications of uremia are rarely seen in patients with kidney disease now that treatment with dialysis and renal transplantation is possible and initiated relatively early.

NEUROMUSCULAR DISTURBANCES

The neuromuscular disturbances occurring in patients with advanced kidney disease were some of the earliest clinical symptoms described in uremia (66). The initial symptoms are mild and consist of emotional lability, insomnia, and a lack of facility in abstract thinking. If the uremic state is allowed to progress, more striking changes are noted, consisting of increased deep tendon reflexes, clonus, asterixis, and stupor, which progress to coma, convulsions, and death.

Uremic neuropathy is another major and potentially disabling complication of chronic kidney disease. The earliest feature is the restless legs syndrome, in which the patient has a tendency to avoid inactivity of the lower extremities because of a sensation of numbness. This syndrome is followed by a sensory neuropathy characterized by paresthesia and hypalgesia, especially of the feet. In the most severe cases, a motor neuropathy also may occur. Typically, there is symmetrical involvement of the lower extremities, which is more severe distally and usually is manifested initially by bilateral footdrop (66). Uremic neuropathy occasionally can progress rapidly to a state of total quadriplegia. Histologically, the damage in the peripheral nerve occurs in the distal portion of the medullated fibers and involves a loss of myelin. For unknown reasons, the motor neuropathy is much more common in males than in females.

SKELETAL ABNORMALITIES (RENAL OSTEODYSTROPHY)

Other major causes of disability in chronic kidney disease, especially in children, are abnormalities in the skeletal system (Table 11-5). Growth is markedly retarded in children with kidney disease. The reasons are not well understood, but there is evidence that dialysis, especially chronic cyclic peritoneal dialysis, improves the growth rate. A high caloric and protein intake also may be helpful. Even with these measures, however, children on dialysis rarely grow normally. The use of corticosteroids after

kidney transplantation also is associated with growth retardation. Recombinant human growth hormone has been used with considerable success to increase height velocity in uremic children and those who have received transplants (67).

Table 11–5 Characteristics of Renal Osteodystrophy

	High Turnover	Low Turnover
Parathyroid hormone	Increased	Decreased
Alkaline phosphatase	Increased	Normal
Osteocalcin	Increased	Normal
Calcium	Variable	Can be increased
Phosphorus	Increased	Normal or increased
DFO stimulation test	Normal	Normal (adynamic) Elevated delta (aluminum OM)
Skeletal radiographs	Resorption, sclerosis	Normal
Symptoms	Usually asymptomatic unless very severe disease	Asymptomatic (dynamic) Symptomatic (aluminum OM)

DFO, deferoxamine; OM, osteomalacia.

Severe rickets with resulting deformities and disability can develop in children with advanced chronic kidney disease. The typical radiographic feature of rickets is an irregular and fragmented line that separates the metaphysis from the growth cartilage (Fig. 11-3). The space separating the metaphyseal line and epiphyseal nucleus is widened, and the epiphyseal center appears late. Although this radiographic finding is classic of vitamin D-deficient rickets, uremic children with this finding characteristically show histologic changes of hyperparathyroidism rather than osteomalacia. Nutritional vitamin D and calcium therapy may be effective in correcting this abnormality.

The most common skeletal disturbance found in adults with advanced chronic kidney disease is hyperparathyroid bone disease, which is characterized by increased osteoclastic bone resorption. Bone histomorphometry performed in 60 nondialyzed uremic patients revealed

that >80% showed evidence of hyperparathyroid bone disease (68). Only one patient in this series had histologic evidence of osteomalacia, and this patient was an alcoholic with chronic pancreatitis, suggesting an etiology other than uremia. The parathyroid glands may be markedly hyperplastic, and PTH levels are increased. Characteristically, there are few symptoms, and the diagnosis is made by finding the typical radiographic features of subperiosteal resorption. In Figure 11-4 are shown the subperiosteal resorption in the phalanges and the "salt-and-pepper" pattern in the skull of a patient with severe secondary hyperparathyroidism associated with advanced kidney disease. Such patients occasionally develop large osteoclastic tumors (brown cysts) in the skeleton around weight-bearing areas, as shown in Figure 11-5A. In these conditions, parathyroidectomy is indicated, following which there usually is dramatic healing of the cyst (Fig. 11-5B). The hyperparathyroid state usually resolves following renal transplantation (69). On occasion, however, persistent hypercalcemia that is endangering the integrity of the kidney graft may necessitate parathyroidectomy.

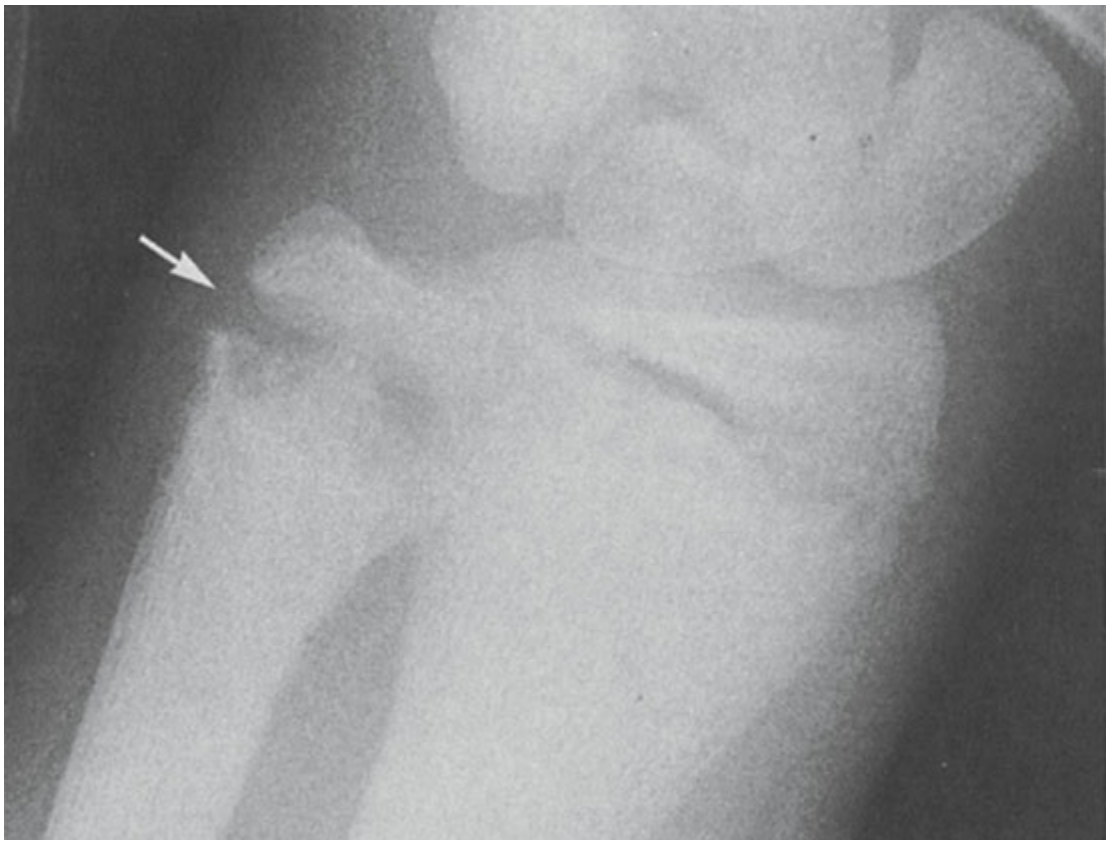

Figure 11–3 Radiographic features of rickets. The metaphysis of the ulna is fragmented and irregular, and the space separating the metaphysis from the epiphyseal nucleus is widened (*arrow*).

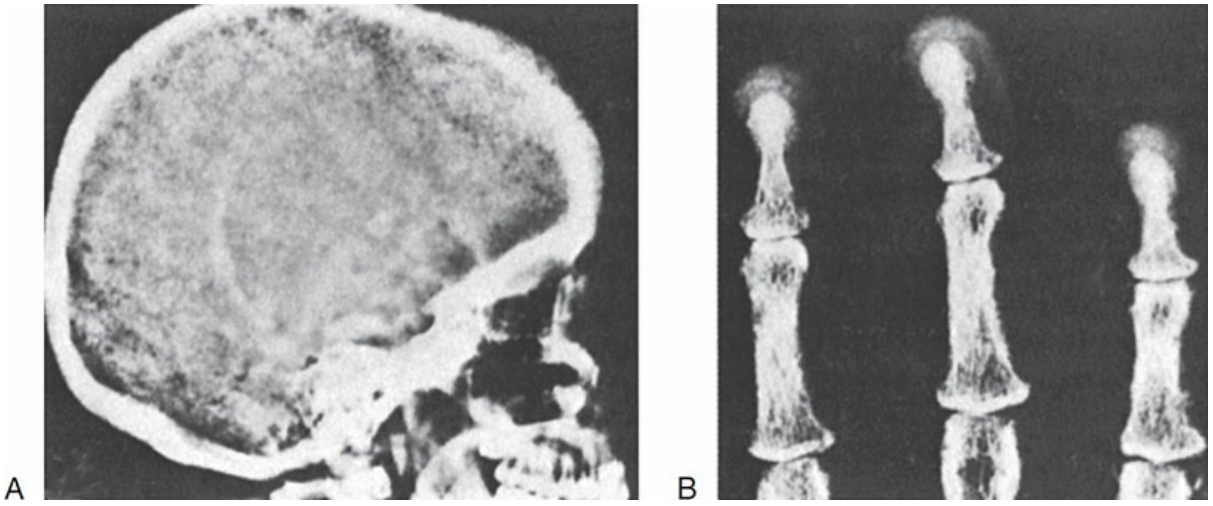

Figure 11–4 (**A**) Typical radiographic features of severe secondary hyperparathyroidism involving skull (note: *salt-and-pepper pattern*). (**B**) Phalanges with lacy subperiosteal reabsorption.

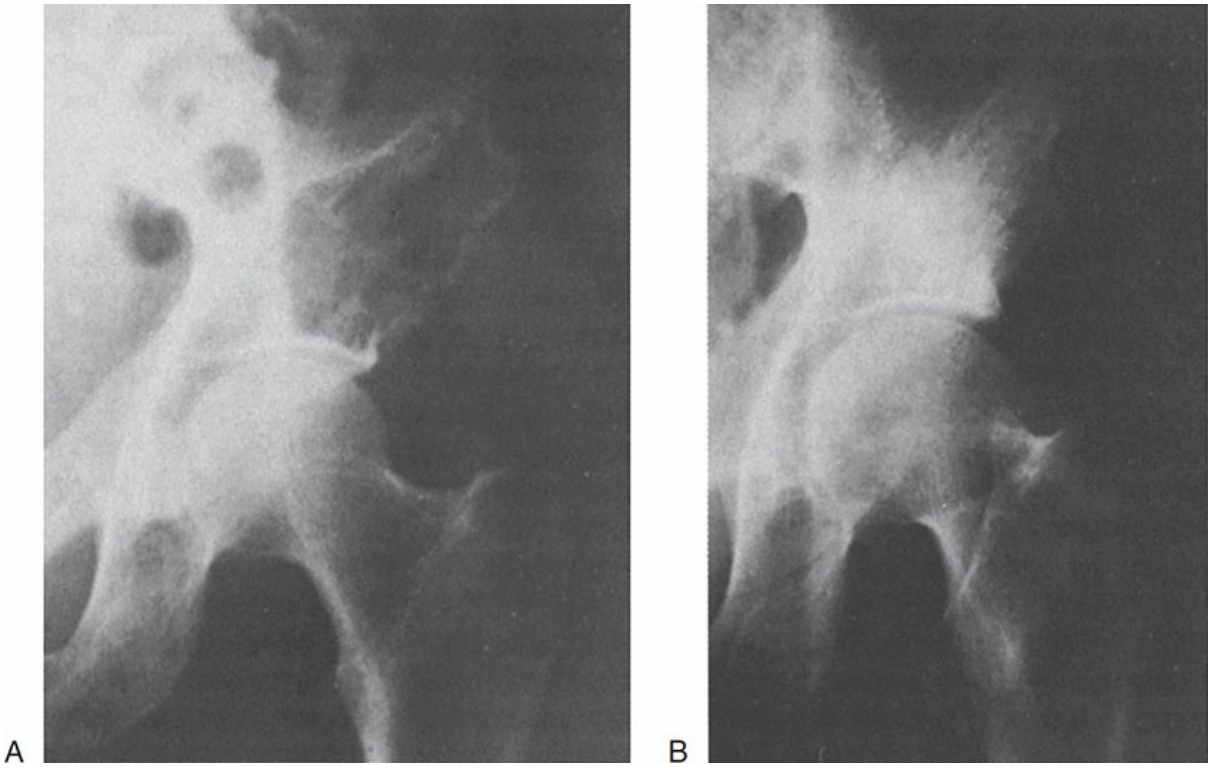

Figure 11–5 (**A**) Biopsy-proven osteoclastic tumor (*brown cyst*) in the left ileum. (**B**) Appearance 1 year after parathyroid surgery. Healing of the cyst is essentially complete as judged radiologically.

With the advent of chronic hemodialysis, osteomalacia has been noted with increased frequency in uremic patients. This disease is characterized by bone pain, fracturing bone disease, and proximal myopathy. Unlike other types of bone disease, osteomalacia is unresponsive to any vitamin D analogs.

Recently, a third type of bone disease has been described, called adynamic bone disease (70). This is a histologic diagnosis showing lack of bone formation and resorption. There are usually no clinical findings, and there are questions about therapy and the association with vascular calcification (71,72).

METASTATIC CALCIFICATION

A serious complication associated with chronic kidney disease is metastatic calcification. Three distinct types of metastatic calcium phosphate deposits have been described in uremic patients. The specific mechanisms responsible for the development of these deposits have not been well delineated, and it is possible that all three have different pathogenic mechanisms.

One of the most potentially devastating forms of metastatic calcification is vascular calcification. An example of diffuse calcification in the arterial vessels of the hand of a patient with advanced kidney disease appears in Figure 11-6. This vascular calcification can affect virtually any medium-sized artery in the body and can cause severe vascular insufficiency with the production of gangrene of the extremities (73) and ischemic ulcerations of the skin and gastrointestinal tract. Although improvement occasionally is observed following renal transplantation, in general, this vascular calcification persists after renal transplantation or parathyroidectomy. Histologic evidence of vascular calcification occurs even in young individuals with uremia, and by age 50 years, radiographic evidence of vascular calcification is present in almost 100% of uremic patients (74). It is felt that vascular calcification results from an accelerated aging process of the vessels in the uremic state.

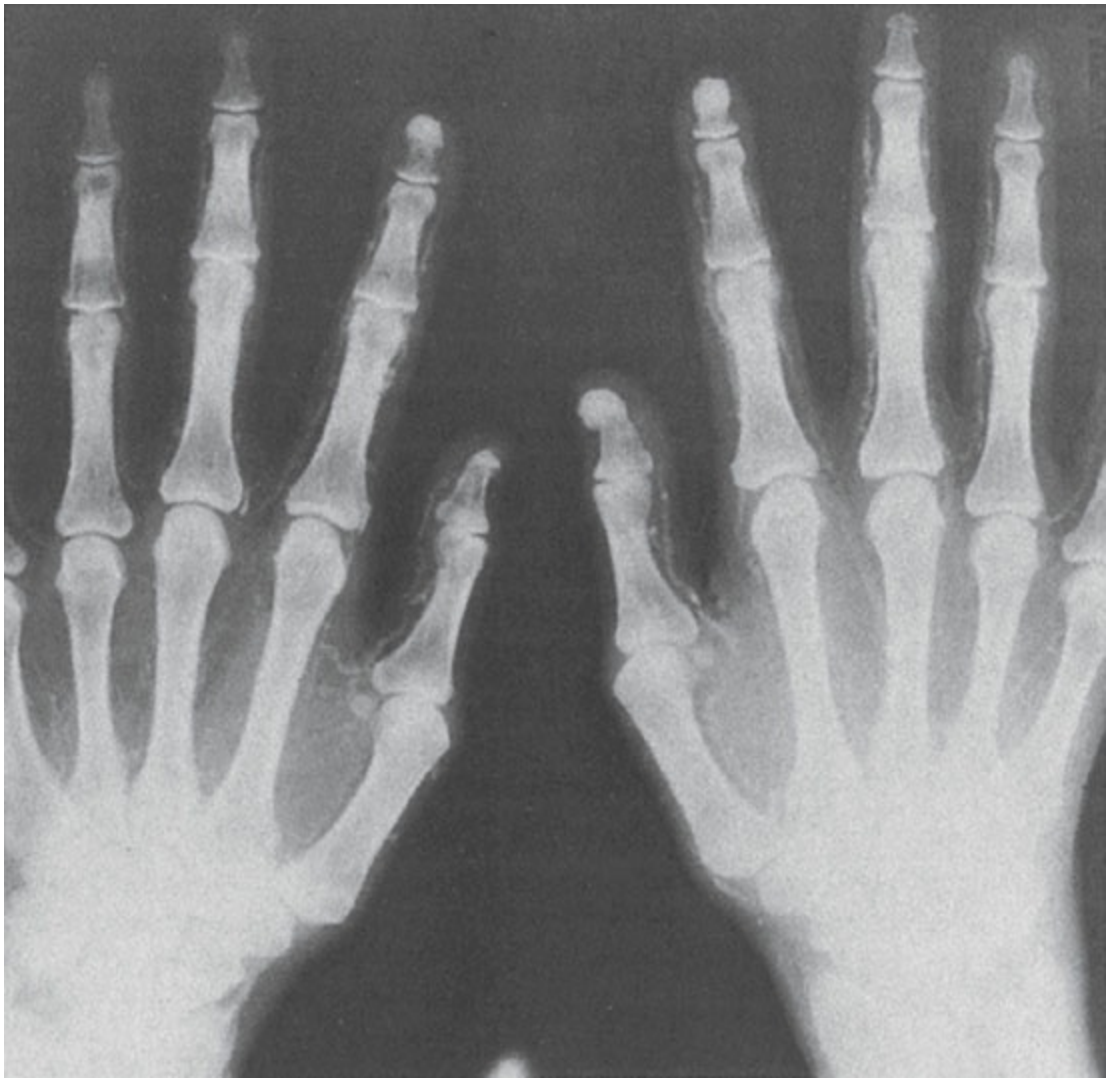

Figure 11–6 Extensive vascular calcification involving the digital arteries.

The second variety of calcium phosphate deposit is felt to result from hyperphosphatemia. This is based on the fact that these deposits can be rapidly mobilized by reducing the serum phosphorus and thus the calcium phosphate product by dialysis, the use of phosphate-binding antacids, or transplantation (69,75). There is a direct relationship between elevated serum phosphorus levels and increased mortality risk. These may be related to metastatic calcification (precipitation of calcium phosphate crystals in undamaged soft tissue) as a result of elevated serum phosphorus, and PTH. These deposits occur in three forms. Conjunctival calcification causes a redness and gritty feeling in the eyes. Periarticular calcification occurs over pressure points and around joints (Fig. 11-7). The major symptom associated with these deposits is a limitation of joint movement because of the size of the deposits. The second type of deposit resulting from hyperphosphatemia is acute arthritic episodes secondary to

hydroxyapatite crystal deposition in the synovium and joint fluid.

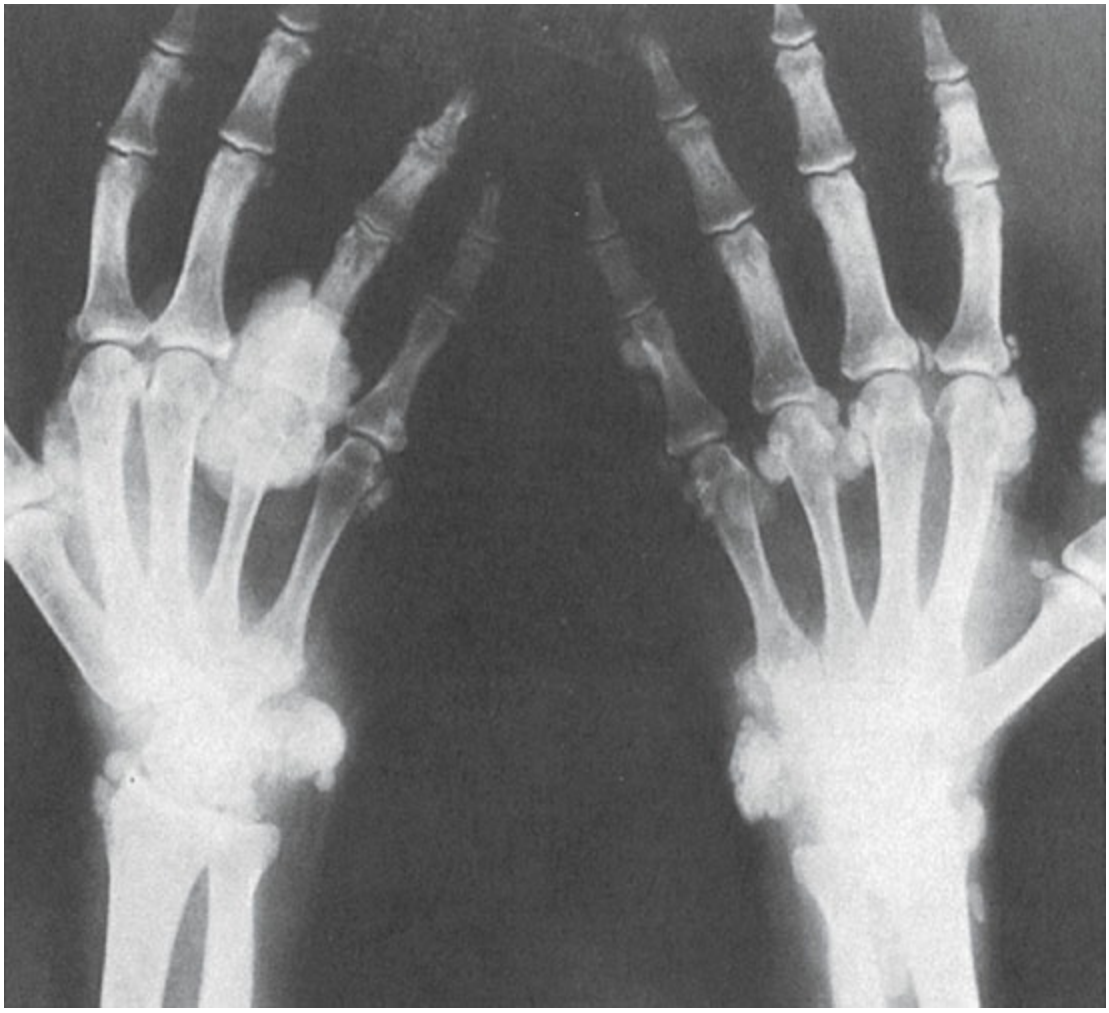

Figure 11–7 Periarticular calcium phosphate deposits (tumoral calcification) in a patient with advanced renal failure.

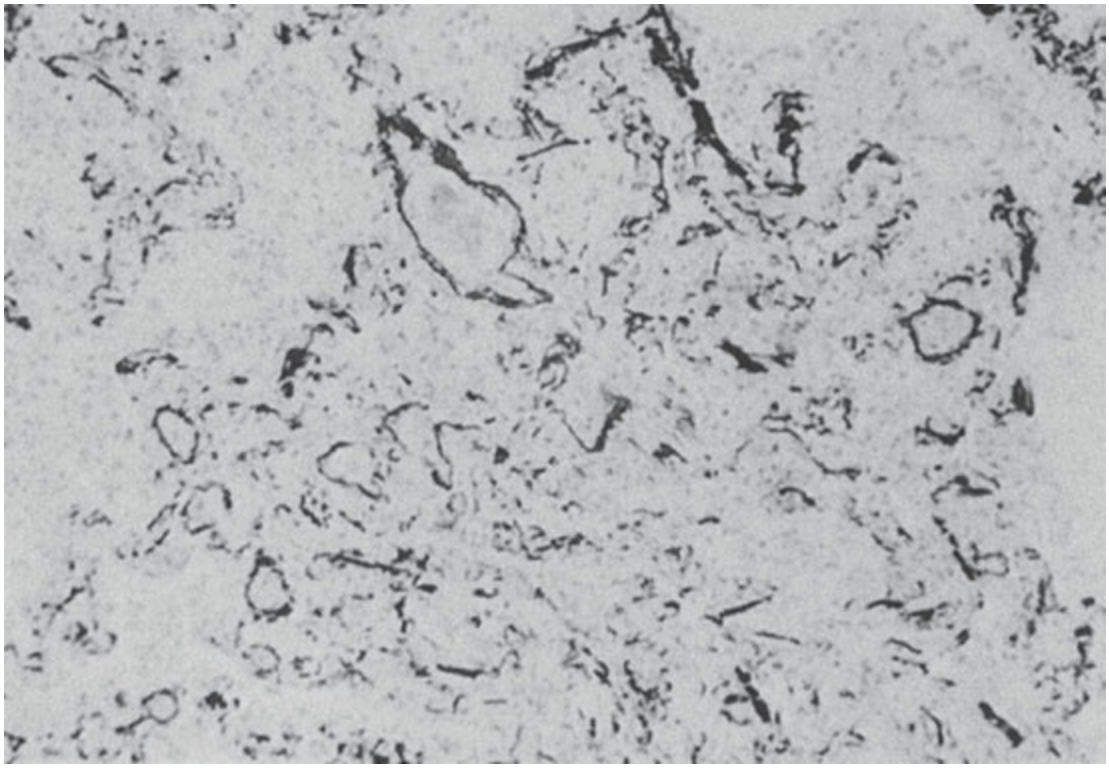

Figure 11–8 Extensive calcification of the lung. The dark staining material (von Kossa stain) present in the alveolar septa and walls of the small arteries is the calcium phosphate deposit.

The final type of calcification found in uremic patients is visceral calcification, which occurs in the lung, skeletal muscle, and myocardium. This is an amorphous calcium phosphate deposit that has markedly different chemical and thermochemical properties from the other two types of calcium phosphate deposits. The vascular and hyperphosphatemic-induced calcifications appear to consist of hydroxyapatite, whereas visceral calcifications have the thermochemical properties of whitlockite. In cardiac calcification, the deposits occur initially in the conducting system and may cause severe arrhythmias. With far-advanced cardiac calcification, however, there is extensive involvement of the entire myocardium, and death may result from a low cardiac output state. When calcification occurs in the lung, it characteristically causes a fibrous response in the small arteries and alveolar septa (Fig. 11-8). This results in restrictive and diffusion abnormalities and can lead to hypoxemia. The pathogenic mechanism responsible for the development of these visceral calcium phosphate deposits is not well understood. Furthermore, it is unknown whether once present they can be mobilized by either transplantation or reduction in the plasma calcium or phosphorus level.

Generalized pruritus is an extremely bothersome complication of chronic kidney disease. Some authors have suggested that high skin

calcium content may be responsible for it (76,77). However, it is possible that skin calcium content also may be secondarily increased as a result of the scratching. Parathyroidectomy has been used in a few cases as a method of treatment of pruritus. However, the majority of patients can be adequately controlled with less radical forms of treatment such as local lubrication, cyproheptadine (Periactin), and reduction in the serum phosphorus with phosphate-binding gels or intensified dialysis.

Calciphylaxis

Calciphylaxis is characterized by calcification of small- and medium-sized blood vessels of the skin and subcutaneous tissue. It is a rare but serious disorder that occurs in uremic patients (78,79). It is manifested by painful erythematous subcutaneous nodules and plaques on the trunk, buttocks, or proximal extremity. Lesions finally progress to necrotic ulcer. Septicemia is the primary cause of death. The pathogenesis of calciphylaxis is still unclear. Hyperparathyroidism, vitamin D supplementation, and hyperphosphatemia all are related to calciphylaxis, but none of these factors has been defined as a specific cause. High plasma levels of PTH, phosphate, and calcium are not very helpful with the diagnosis because they may not always be present. The diagnosis can be confirmed by a skin biopsy. Early diagnosis and treatment is important given the high mortality in this condition. Tight control of plasma calcium and phosphorus concentrations, avoidance of trauma, and aggressive wound care are all important. Parathyroidectomy is suggested in dialysis patients with plasma intact PTH levels higher than 200 pg/mL, but its beneficial role is still controversial (80,81).

IMMUNOLOGIC ALTERATIONS

The immune response also is blunted in the majority of patients with advanced kidney disease, as manifested by depressed delayed hypersensitivity, prolonged survival of skin allografts, and reduced lymphocyte response to phytohemagglutinin (82). The uremic patient also appears to have a poorer immune response to some infections than nonuremic subjects. Perhaps this is best exemplified by the frequent occurrence of positivity for hepatitis B surface antigen (HBsAG) in patients with advanced chronic kidney disease (83). Although these patients usually do not manifest clinical symptoms of hepatitis, they become chronic carriers of the hepatitis-associated antigen. This

continuing positivity for HBsAG recently has been related to low interferon levels in uremic patients (84).

Most studies show that humoral immunity is normal otherwise. It has recently been shown that $1,25(OH)_2D_3$ modulates the proliferation, differentiation, and immune function of lymphocytes and monocytes. On the basis of this finding, it has been suggested that part of the compromised immune function in uremia may be a consequence of the low $1,25(OH)_2D_3$ present in this state (85).

OTHER METABOLIC DISTURBANCES

Finally, a variety of generalized metabolic disturbances are present in the uremic patient. As a result of anorexia, nausea, poor dietary intake, and occasional vomiting, many patients with advanced kidney disease present in a chronic state of negative nitrogen balance and protein–calorie malnutrition.

Approximately 70% of uremic patients present with glucose intolerance. In contrast to patients with diabetes mellitus, the fasting blood sugar of uremic patients is normal, but postprandial glucose levels are increased (86). Plasma insulin levels in response to intravenously administered glucose are normal or even accentuated, thus suggesting that the glucose intolerance is a result of peripheral resistance to insulin. There is evidence that this abnormality in glucose metabolism may be improved by hemodialysis. Although glucagon levels are known to be increased in patients with advanced kidney disease, dialysis may improve glucose intolerance without altering glucagon levels.

A number of patients with kidney disease have increased levels of serum triglycerides and reduced high-density lipoprotein cholesterol. Whether this disturbance has any clinical importance has not yet been established, but there is evidence of acceleration of atherosclerosis in patients treated with chronic intermittent hemodialysis. In addition, experimental evidence suggests that abnormal lipid metabolism may contribute to the progression of renal disease. However, there is growing skepticism regarding the benefits of lowering serum cholesterol in the chronic dialysis patient population. This uncertainty is further strengthened by two clinical trials examining the use of statins in dialysis patients. Whereas statins have been consistently shown to reduce cardiovascular death and other cardiovascular events in non–chronic kidney disease patients, neither the atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis (4D) trial (a placebo-controlled randomized trial

of atorvastatin in 1,255 maintenance HD patients with type 2 diabetes) (87) nor the Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events (AURORA) trial (a placebo-controlled randomized trial of rosuvastatin in 2,667 patients) (87) showed the anticipated reduction in cardiovascular death, nonfatal MI and nonfatal stroke, despite >40% reductions in serum low-density lipoprotein cholesterol levels.

Pathogenesis of the Uremic Syndrome

Although the metabolic consequences of uremia have been reasonably well defined, the specific uremia toxins responsible for most of these metabolic alterations have not been identified. It is apparent, however, that certain organic compounds, hormonal alterations, and inorganic substances that are affected by the uremic state may cause a number of defects (Table 11-6).

ORGANIC COMPOUNDS

Most studies of organic compounds have been directed at determining the toxicity of the various nitrogenous waste products, which consist of urea, ammonia, guanidine, guanidinosuccinic acid, and methylguanidine. Because urea is characteristically elevated and can be correlated with the severity of impairment of kidney function, a large number of studies have been directed at determining the role of urea in producing the uremic symptomatology. However, the results of most studies have been disappointing, and at present the only abnormalities that have been suggested as resulting at least in part from urea retention are nausea, anorexia, uremic stomatitis, and possibly uremic colitis.

Table 11–6 Compounds Incriminated As "Uremic Toxins"

Byproducts of protein and amino acid metabolism
Urea—80% of total (excreted nitrogen)
Guanidino compounds
Guanidines
Creatinine/creatine
Other nitrogenous substances
Polyamines

- Myoinositol
- Phenols
- Benzoates
- Indoles

Advanced glycation end products
Inhibitors of ligand–protein binding
Glucuronoconjugates and aglycones
Inhibitors of somatomedin and insulin action
Middle molecules
Parathyroid hormone
β_2-Microglobulin

Guanidine has been found to be increased in the blood of uremic patients. When injected into laboratory animals, guanidine produces muscle twitching, hyperexcitability, paresis, and convulsions. However, recent studies in uremic patients have failed to show a correlation between high guanidine levels and central nervous system symptoms (88).

Another potentially toxic organic compound is myoinositol, a natural constituent of food that also is synthesized in vivo. The concentration of this compound in plasma and cerebrospinal fluid is increased in uremia. Experimentally, myoinositol has been shown to be a neurotoxin, and therefore it has been suggested that this compound might be involved in the pathogenesis of uremic neuropathy (89). Plasma cyclic adenosine monophosphate (cAMP) levels have also been found to be increased in uremic patients (90). Plasma cAMP levels have been found to correlate inversely with platelet aggregation, and cAMP has been shown to inhibit platelet aggregation in vitro (90). Thus, cAMP may be partly responsible for the altered platelet function in uremia.

Possibly the strongest evidence that some of the nitrogenous waste products may be toxic in uremic patients are the studies of Walser et al. (91). In their studies, a number of patients were placed on diets extremely low in nitrogen to reduce the production of nitrogenous waste products. The diets were supplemented with the ketoanalogs of the essential amino acids. These ketoanalogs were used to supply the carbon skeletons for the essential amino acids and thus to prevent the breakdown of tissue protein. There was marked improvement in the patients' sense of well-being and possibly in some other abnormalities associated with the uremic syndrome. These results deserve further study, although they are not conclusive in demonstrating that nitrogenous waste products have a role in producing

some uremic symptoms.

Another organic toxin recently identified in dialyzed uremic patients is the protein β_2-microglobulin. Normally the kidney is responsible for the elimination of this protein, which has a molecular weight of 11,800 Da. The 150 to 200 mg produced each day are filtered by the glomerulus and catabolized in the proximal tubule. Elimination of this protein is prevented and retained in advanced kidney disease, increasing plasma levels up to 50-fold and causing it to be deposited in tissues. The protein is deposited in tissues as amyloid, with its deposition being present largely in joint capsules, synovial membranes, the carpal tunnel, subchondral bone, tendons, intervertebral discs, and bone marrow (92). Clinical symptomatology from β_2-microglobulin deposition includes carpal tunnel syndrome, bone cysts, destructive spondyloarthropathy, effusive arthritis, scapulohumeral, and periarthritis. These complications usually are seen in dialyzed uremic patients, probably because it requires a minimum of 6 years of almost total loss of kidney function for enough β_2-microglobulin to be deposited to cause any clinical disturbances. Once deposited, it appears that this amyloid is not mobilized, even following reinstitution of kidney function with a successful renal transplant.

HORMONAL ALTERATIONS

Hormonal alterations occur in uremia as a result of four mechanisms. First, the diseased kidney may be unable to produce certain hormones normally, such as EPO and $1,25(OH)_2D_3$ (93). Second, the kidney normally excretes or degrades a variety of hormones: growth hormone, prolactin, luteinizing hormone, gastrin, insulin, glucagon, and PTH. Levels of these hormones may be increased in patients with chronic kidney disease because of lack of ability of the diseased kidney to metabolize or excrete these substances. Third, the diseased kidney may cause increased renin secretion as a result of ischemia, which in turn increases angiotensin and aldosterone production. The final mechanism for hormone alteration in uremia is the *trade-off hypothesis* (94).

The various hormonal alterations present in the uremic state may result in a variety of clinical disturbances (Table 11-7). However, the mere finding of increased levels of immunoreactive hormones does not necessarily mean that they are biologically active. That some of these hormones lack biologic activity is suggested by the fact that gastrin levels are not correlated with basal acid secretion in uremic patients. Studies also

suggest that the measured growth hormone and possibly glucagon are not biologically active. Furthermore, it has been shown that the largest fraction of the elevated levels of immunoreactive PTH is the inactive *C*-terminal, which is normally disposed by the kidney.

Elevated PTH levels have been incriminated in the pathogenesis of numerous abnormalities found in the uremic state, including neuropathy, encephalopathy, anemia, pruritus, impotence, and carbohydrate and lipid alterations (95). Although PTH has been shown in vitro to increase red cell osmotic fragility, decrease erythropoiesis, and decrease myocardial function and in vivo to be associated with increased brain calcium content and electroencephalographic (EEG) alterations, it is still unclear what role PTH plays in the uremic symptomatology found in humans.

Trade-off Hypothesis

Bricker (94) has proposed another conceptual approach to the pathogenesis of uremia, the so-called trade-off hypothesis. This theory suggests that with the progressive destruction of nephrons, a number of adaptive mechanisms are brought into play to allow the remaining nephrons to maintain normal body homeostasis. The adaptive changes that occur to maintain sodium balance in the patient with advanced kidney disease have been used as an illustrative example of the trade-off hypothesis. Approximately 0.5% of the filtered load of sodium is excreted with a normal sodium intake of 120 mEq/day and a normal GFR. However, on a similar sodium intake, when GFR has fallen to 2 mL/minute, ˜30% of the filtered load of sodium must be excreted to maintain sodium balance. The assumption is made that there is a substance that inhibits the tubular reabsorption of sodium, possibly a natriuretic hormone. It is further suggested that this inhibitor is present in high concentrations in the uremic patient’s serum and that it may affect a variety of cellular transport systems and in turn lead to functional changes in other organs and organ systems. The trade-off is that the effects of such a hormone would be beneficial for the kidney and sodium balance but might cause some of the symptoms of uremia by inhibiting transport systems in other organs.

Table 11–7 Hormonal Alterations in Uremia

Hormone	Potential Metabolic Consequences

Increased	
Prolactin	Lactation
Luteinizing hormone	Gynecomastia
Gastrin gastritis	—
Renin–angiotensin–aldosterone	Hypertension
Glucagon	Glucose intolerance
Growth hormone	—
Parathyroid hormone	Osteitis fibrosis
Decreased	
1,25-$(OH)_2D_3$	Osteitis fibrosa
Erythropoietin	Anemia
Somatomedin	Decreased linear growth
Testosterone	Impotence
Follicle-stimulating hormone	Impotence

The increased PTH levels found in patients with chronic kidney disease have been suggested to be another example of a trade-off phenomenon. In this instance, it is proposed that phosphate excretion is reduced and serum phosphorus rises as nephrons are lost, even early in the course of kidney disease. The elevated serum phosphorus then causes serum calcium concentration to fall, an effect that leads to increased PTH release. The increased parathyroid activity then is associated with a decrease in tubular reabsorption of phosphorus, which results in increased phosphorus excretion and returns the serum phosphorus to near-normal levels. As more nephrons are lost and the amount of filtered phosphate further decreases, higher serum concentrations of PTH are required to maintain phosphorus balance. The trade-off for the maintenance of phosphorus would be the clinical consequences of secondary hyperparathyroidism on the bony skeleton. More recently, studies suggest that hyperphosphatemia per se can stimulate PTH secretion. However, other factors as well are also probably important in the pathogenesis of the hyperparathyroid state. Studies have shown that there is a reduced number of 1,25$(OH)_2D_3$ (calcitriol) receptors on the parathyroid gland in the uremic state (96). Because calcitriol suppresses the synthesis of PTH, the combination of decreased calcitriol receptor number and impaired renal production of 1,25$(OH)_2D_3$ markedly decreases this hormone's inhibitor effect on PTH synthesis (97). Bone resistance to PTH in patients with kidney disease may be an additional cause of secondary

hyperparathyroidism.

INORGANIC SUBSTANCES

The role of inorganic substances in producing some of the uremic symptomatology has received increasing interest. Brain and peripheral nerve calcium levels have been found to be increased in uremic patients. The elevated calcium levels have been associated with impairment of neurologic function, EEG disturbances (98), and reduced motor nerve conduction velocities (98). The finding that the elevated calcium levels and altered neurologic function can be prevented by parathyroidectomy suggests that PTH is involved in the pathogenesis of these disturbances; however, the observation of increased calcium levels in peripheral nerves awaits confirmation.

Phosphorus represents another inorganic toxin. As stated, phosphorus retention probably is quite important in the pathogenesis of the secondary hyperparathyroidism state in uremic subjects (94). Phosphorus retention also is involved in the pathogenesis of metastatic calcification.

On the basis of strong biochemical and epidemiologic studies, it is now firmly established that aluminum intoxication is responsible for major neurologic and skeletal toxicity in uremic patients (99,100). Although aluminum intoxication initially was felt to result only from aluminum-contaminated dialysate, it is now apparent that toxicity can also result from the orally administered, aluminum-containing phosphate-binding gels commonly given to uremic patients. Aluminum-induced neurologic toxicity is characterized by a distinctive speech disturbance, myoclonus, seizures, and dementia. It is progressive, with death usually occurring 6 to 8 months after the onset of the first symptoms (99). The bone disease associated with aluminum intoxication is fracturing osteomalacia, which is resistant to treatment with vitamin D analogs (100). Studies suggest that both encephalopathy and osteomalacia can be cured by chelation of aluminum with deferoxamine. A microcytic hypochromic anemia also attributed to aluminum intoxication has been found to improve following deferoxamine treatment. Thus, aluminum-containing phosphate-binding gels are not routinely used for the management of hyperphosphatemia.

Although zinc deficiency has been implicated as the cause of impotence and anorexia in uremic patients, zinc replacement has given conflicting results, with some investigators finding improvement in potency, smell, taste, and appetite, whereas others find no effect. There is little evidence that other trace element alterations are responsible for any

additional symptomatology found in uremic patients.

Thus, it appears that a variety of toxins are responsible for the array of clinical signs and symptoms of the uremic state and that a number of different pathogenetic mechanisms are involved. However, only a relatively small number of toxins and mechanisms have been identified or defined to explain the wide spectrum of uremic symptomatology.

Progression of Kidney Disease

It has been well documented that when a critical loss of nephron population occurs from a variety of different causes, the remaining renal tissue ultimately is lost, resulting in ESRD. The most classic example of this phenomenon in humans is oligomeganephronia (101). This is a congenital disease in which a child is born with a markedly reduced number of nephrons. The nephrons that are present are greatly hypertrophied. However, these hyperfunctioning nephrons undergo spontaneous destruction over the first few years of life, and the child usually dies of uremia by 3 or 4 years of age. Other examples supporting the concept that secondary or compensatory changes resulting from loss of renal mass may promote further injury are unilateral renal agenesis (102) and severe reflux nephropathy, both of which may develop glomerulosclerosis and progress to ESRD.

Table 11–8 Secondary Factors Leading to the Progression

Intraglomerular hypertension and glomerular hypertrophy
Proteinuria
Tubulointerstitial disease
Hyperlipidemia
Phosphate retention
Metabolic acidosis
Iron toxicity
Uremic toxins
Increased prostanoid metabolism

The compensatory mechanisms resulting from loss of kidney function that mediate this injury have not been totally defined, although altered intrarenal hemodynamics (103) and systemic hypertension (104) currently

are felt to be of major importance. Studies have shown that when renal mass is surgically reduced, glomerular plasma flow, intraglomerular hydraulic pressure, and GFR of the remaining nephrons are markedly increased. These altered intrarenal hemodynamic events may lead to glomerular injury, resulting in glomerular sclerosis and functional deterioration. Any injury or disease that reduces nephron population appears to cause a compensatory afferent arteriolar dilatation, which allows the glomerular capillary bed to be exposed to increased hydraulic pressure with resulting injury. This is supported by the fact that hypertension is especially injurious to the glomeruli of a diseased kidney and that treatment of hypertension markedly reduces the rate and severity of glomerular injury in kidney diseases such as diabetic glomerulosclerosis and experimental glomerulonephritis. The evidence for the harmful effects of altered intrarenal hemodynamics on the diseased kidney is substantial. However, other secondary or compensatory changes, such as compensatory glomerular hypertrophy, may result in glomerular injury (Table 11-8). Similarly, increased intrarenal energy requirement (105–108), renal parenchymal calcification, or tubule fluid iron also may be important in promoting tubulointerstitial injury in a diseased kidney (109,110).

Reversible Factors Compromising Kidney Function

Kidney function in chronically diseased kidneys can be further compromised by a number of potentially reversible factors (Table 11-9).

Table 11–9 Reversible Factors Responsible for Renal Function Deterioration

Infection
Urinary tract obstruction
Extracellular fluid volume depletion
Nephrotoxic agents
Congestive heart failure
Hypertension
Pericardial tamponade
Hypercalcemia
Hyperuricemia >15–20 mg/dL

VOLUME DEPLETION

The most common reversible factor that can cause rapid deterioration of kidney function is depletion of the ECF volume. If ECF volume depletion develops in a patient who already has compromised kidney function, a vicious cycle of events may ensue. The diminished kidney function that accompanies ECF volume depletion may cause worsening of the azotemic state. With the increased azotemia, nausea and vomiting may occur, leading to more volume depletion, further compromising GFR, and intensifying the uremia state, thus repeating the cycle. The diagnosis of severe volume depletion usually is obvious, because the patient will demonstrate tachycardia, postural hypotension, a dry furrowed tongue, and loss of skin elasticity. At an earlier stage of volume depletion, however, physical findings may be minimal. A history of overzealous treatment with potent diuretic agents or stringent salt restriction may suggest the diagnosis of modest volume depletion in patients with chronic kidney disease who have had recent deterioration of kidney function. It is frequently necessary to treat the patient with salt supplementation to determine whether kidney function will improve. A weight gain of 1 to 3 kg generally constitutes a reasonable indication of ECF volume expansion.

INFECTION AND OBSTRUCTION

Because many urinary tract infections are asymptomatic, a urine culture should be obtained on the initial evaluation of patients with chronic kidney disease. To prevent the introduction of a bacterial infection, instrumentation and indwelling catheterization of the urinary tract also are best avoided in such patients. Another potentially reversible cause of kidney disease is urinary tract obstruction. Obstruction can be excluded with about 95% confidence by performing ultrasound studies; therefore, retrograde pyelograms are seldom necessary, even when the patient has severely compromised kidney function. Ultrasound also gives valuable information with regard to the presence of two kidneys as well as kidney size. A postvoid residual urine as assessed by a straight catheterization after maximal voiding effort allows exclusion of important bladder neck obstruction (>50 mL of residual urine). A flat-plate radiograph of the abdomen with nephrotomograms can also help establish kidney size and exclude the presence of radiopaque calculi.

NEPHROTOXIC AGENTS

Nephrotoxic agents are another potential cause of reversible acute kidney injury. The most common are the antimicrobials and antitumor agents, including aminoglycosides, colistin, amphotericin, gallium, and cisplatin. An additional group of agents that can further compromise kidney function in patients with chronic kidney disease is radiocontrast media. Predisposing factors to contrast media-induced kidney injury include diabetes mellitus, advanced age, volume depletion, other nephrotoxic agents such as aminoglycosides, and preexisting kidney disease. Although the use of these agents in patients with chronic kidney disease is not contraindicated, they should be used with the consideration that they can cause permanent as well as reversible changes in kidney function. Because toxicity is somewhat dose dependent, the smallest dose consistent with obtaining an adequate study should be administered. In addition, the patient's volume status should be optimized with saline at the time of the contrast media administration (111).

PHARMACOLOGIC REDUCTION IN FUNCTION

Recently, two other drug classes, ACE inhibitors and cyclooxygenase inhibitors (112,113), have been shown to decrease kidney function acutely and reversibly in patients with underlying kidney disease. NSAIDs cause this effect by inhibiting renal prostaglandins and their vasodilatory effect, which causes a reduction in renal blood flow and GFR. The ACE inhibitors exert their effect by inhibiting angiotensin II constriction of the glomerular efferent arteriole. This causes a reduction in filtration pressure and, in turn, GFR. The effects of these agents on GFR are most commonly seen when renal arterial flow is reduced as a consequence of volume depletion, congestive heart failure, cirrhosis, bilateral renal artery stenosis, where filtration pressure is largely being maintained by angiotensin II-induced efferent arteriolar constriction. The effects of both the cyclooxygenase inhibitors and converting enzyme inhibitors on reducing GFR are rapidly reversed by discontinuing their administration. Therefore, when indicated, the use of these drugs in patients with chronic kidney disease is not universally contraindicated.

CARDIOVASCULAR EFFECTS

Congestive heart failure in the uremic patient can result from a variety of causes, such as atherosclerosis, hypertension, and fluid overload.

Treatment of congestive heart failure may improve kidney function. Blood pressure should be normalized by fluid removal, antihypertensive agents, or both. Diuresis usually can be accomplished with large doses of furosemide; however, when kidney disease is severe, dialysis may be indicated to remove the excess body fluid. Digoxin dosage has to be modified in patients with compromised kidney function because digoxin is largely excreted by the kidney.

The possibility of uremic pericarditis with associated pericardial effusion and tamponade should be considered in patients with far-advanced kidney disease. The clinical features of this diagnosis consist of increased jugular venous pulsations with a paradoxical inspiratory accentuation (Kussmaul sign), pulsus paradoxus, and a reduction in systemic blood pressure and pulse pressure. A pericardial friction rub may or may not be present. The paradoxical pulse may not be present with an extremely severe cardiac tamponade, and the only finding may be a reduction in blood and pulse pressures. The diagnosis is easily confirmed by ultrasound or blood-pool scanning. Pericardiocentesis may be lifesaving; in addition, by relieving the tamponade, it may improve cardiac output and kidney function. Finally, treatment of severe hypercalcemia, hypokalemia, and hyperuricemia also may lead to improvement in kidney function.

Decreasing Rate of Functional Deterioration

DIABETIC RENAL DISEASE

Diabetic nephropathy is characterized by persistent albuminuria (>300 mg/24 hours), a raised arterial blood pressure, and a relentless decline in GFR (114). Diabetic nephropathy rarely develops before 10 years after the onset of disease in type 1 diabetes, whereas ˜3% of newly diagnosed type 2 diabetes patients have overt nephropathy (8). Nephropathy owing to type 2 diabetes accounts for the increasing number of patients with kidney failure in the past decade. Several factors have been identified that might either prevent the development of diabetic renal disease or slow its progression to ESRD.

METABOLIC CONTROL

A multicenter study involving 1,441 patients with type 1 diabetes showed

that intensive insulin therapy delayed the onset and slowed the progression of diabetic retinopathy, nephropathy, and neuropathy (115). In this Diabetes Control and Complications Trial (DCCT), intensive therapy reduced the occurrence of microalbuminuria by 39% and that of overt albuminuria by 54%. In patients with type 2 diabetes, the UK Prospective Diabetes Study (UKPDS) reported that intensive blood glucose control decreases the risk of microvascular complications, but not macrovascular disease (116). In this study, 3,867 newly diagnosed patients with type 2 diabetes were randomly assigned to intensive treatment with sulfonylureas or insulin, or conventional management with diet. After 12 years of follow-up, there is a decreased rate of doubling serum creatinine with tight blood glucose control.

Further support for the beneficial effect of tight blood glucose control is that transplantation of a kidney in conjunction with a pancreas in patients with ESRD from diabetes has been found to prevent mesangial expansion and thickening of the basement membrane in the transplanted kidney (117,118). These glomerular changes are commonly observed when the kidney alone is transplanted without a pancreas into diabetic patients. As expected, more advanced renal diabetic lesions are not reversible. This raises the possibility of using islet cell transplantation in type 1 diabetes in the absence of diabetic nephropathy (119,120).

RENIN–ANGIOTENSIN SYSTEM AND BLOOD PRESSURE CONTROL

In addition to glycemic control, there also is considerable epidemiologic evidence, suggesting that hypertension plays a significant role in the development and progression of diabetic nephropathy. The long-term antihypertensive treatment in reducing albuminuria and slowing the rate of decline in GFR from 15 to 6 mL/year in male type 1 diabetic patients with overt nephropathy was first described by Mogensen (121). Parving et al. (122) confirmed these observations and demonstrated that early aggressive antihypertensive treatment with metoprolol, furosemide, and hydralazine reduces albuminuria and the decline in GFR, as well as postponing ESRD in young male and female type 1 diabetic patients with nephropathy. The critical role of the renin–angiotensin system in addition to blood pressure control in slowing the progression of kidney diseases was examined in a multicenter study involving over 400 patients with type 1 diabetes using captopril (123). The study demonstrated that the use of ACE inhibitor protected against deterioration of kidney function independent of blood

pressure control. Thus, it is suggested that ACE inhibitors are protective not only as a consequence of blood pressure control but probably more importantly because of their intrarenal effects on decreasing efferent arteriolar constriction, glomerular hydraulic pressure, and reducing proteinuria.

Similar results were also observed with studies in type 2 diabetic patients. The Appropriate Blood Pressure Control in Diabetes trial (ABCD) demonstrated an advantage of an ACE inhibitor over a long-acting calcium channel antagonist with regard to the incidence of MI (124–127). The ABCD trial was a prospective randomized study evaluating the effects of intensive diastolic blood pressure control on the progression of diabetic vascular complications. Two groups were studied separately—a normotensive type 2 diabetic population (diastolic blood pressure between 80 and 90 mm Hg) and a hypertensive type 2 diabetic population (diastolic blood pressure ≥90 mm Hg). In the hypertensive cohort, at comparable blood pressure, cholesterol, glycohemoglobin levels, MIs were decreased with ACE inhibition versus calcium channel blockers as initial therapy. Blood pressure control, either at moderate (mean 138/86 mm Hg) or intensive (mean 132/78 mm Hg) level stabilized kidney function in normoalbuminuric and microalbuminuric patients. Patients with overt diabetic nephropathy however averaged 5 mL/min/1.73 m^2/y decline, thus they may avoid ESRD until their late seventies. Intensive versus moderate blood pressure control decreased all-cause mortality. In the normotensive cohort, intensive (mean 128/75 mm Hg) and moderate (mean 137/81 mm Hg) blood pressure control stabilized creatinine clearance in normoalbuminuric and microalbuminuric patients. However, there was also 5 mL/min/1.73 m^2/y decline in creatinine clearance in patients with overt albuminuria. The intensive blood pressure control group also showed an advantage in slowing the progression of normoalbuminuria to mircroalbuminuria, decreasing the progression of diabetic retinopathy, and decreasing the incidence of strokes. Thus, intervention is most effective before overt albuminuria (7,300 mg/24 hours) in type 2 diabetes.

The UKPDS study also demonstrated that more tight control of blood pressure decreased stroke, diabetic-related death and microvascular complications including retinopathy and nephropathy (128). A substudy of the Heart Outcome Prevention Evaluation Study, known as MICRO-HOPE, found that ACE inhibition with ramipril provided protection against cardiovascular events and attenuated the increase in proteinuria in diabetic subjects with microalbuminuria (129). Moreover, an analysis by

Golan et al. (130) concluded that an ACE inhibitor would be cost effective if it were prescribed for all middle-aged patients with type 2 diabetes, irrespective of their baseline kidney function.

The use of ARBs was recently examined in type 2 diabetic patients. Parving et al. (131) used irbesartan in patients with type 2 diabetes and hypertension who had normal GFR, but early kidney damage as manifested by microalbuminuria. The drug was associated with lower levels of microalbuminuria than the placebo. The GFR fell slightly initially, probably because of hemodynamic action of the ARB, but the long-term trend did not differ among the groups. The diminution of albuminuria indicates potential protection from ongoing kidney damage that would translate into preservation of the GFR in the long term. Two other studies, by Brenner et al. (132) and Lewis et al. (133), also used ARBs losartan and irbesartan, respectively, to study type 2 diabetic patients with established diabetic nephropathy. In these patients, the use of these drugs resulted in a decrease in the combined end point of doubling serum creatinine, progression to ESRD and death in patients receiving the ARBs. In both studies, the benefits could not be explained by the effects of the drugs on blood pressure. In both type 1 and 2 diabetic patients, drugs that block the renin–angiotensin–aldosterone system appear to decrease glomerular capillary pressure, reduce fibrogenesis, and attenuate the proliferative actions of angiotensin and aldosterone (134), thus slowing the progression of chronic kidney disease.

PROTEINURIC KIDNEY DISEASE

In proteinuric kidney disease, accumulating evidence suggests that any means of reducing urinary protein excretion (e.g., dietary protein restriction and ACE inhibitors) may exert a protective effect on kidney function. However, a large multicenter trial carried out in 840 patients with chronic kidney disease showed that a very low-protein diet had a minimal effect on slowing the progress of kidney function deterioration (135). In view of this study as well as poor patient acceptance, dietary protein restriction does not appear to be a feasible or effective means of treating patients with chronic kidney disease because of the potential for inducing protein malnutrition and the expense of dietary management and amino acid supplementation. However, there is increasing evidence that ACE inhibitors are effective not only in diabetic kidney disease but also in any proteinuric renal disease (136,137). Furthermore, results from the MDRD (135) and a ramipril trial (138) are compatible with the importance of the

initial antiproteinuric response to antihypertensive therapy. In the MDRD study, for each 1 g/day reduction in protein excretion in the first 4 months, the rate of decline in GFR fell by 0.9 to 1.3 mL/min/y. The fall in proteinuria was related to the blood pressure, being more prominent in those with more aggressive blood pressure control. In the ramipril trial, the rate of decline in GFR correlated inversely with the degree of proteinuria reduction among patients excreting >3 g protein/day. However, there are studies in which these ACE inhibitors have decreased proteinuria and yet worsened kidney function. Although calcium channel blockers in general do not decrease proteinuria to the degree of ACE inhibitors, some studies using animals have shown protective and additive effects on proteinuria with ACE inhibitors and nondihydropyridine calcium channel blockers (139–141). ACE inhibitors have only been shown to have a protective effect in proteinuric kidney disease. Otherwise, any protection exerted in nonproteinuric kidney disease is probably only a result of blood pressure control. Studies estimate that 10% to 15% of patients may not tolerate these drugs because of their causing chronic coughing or hyperkalemia. To avoid the side effect of coughing, an ARB can be used instead. The optimal antiproteinuric effect with therapy is not clear. A target reduction of at least 30% to 40% from baseline urinary protein levels has been recommended because this response appears to correlate with a lowering of intraglomerular pressure in experimental settings.

HYPERTENSION

Increasing evidence also suggests that the control of hypertension can have a major effect on reducing the progression of chronic kidney disease in patients with essential hypertension as well as primary kidney disease. African Americans are especially at risk of ESRD from hypertension (14–17). Initial studies showed that patients with chronic kidney disease and a diastolic pressure <90 mm Hg had better preservation of GFR than hypertensive patients. However, these uncontrolled observations alone do not exclude the possibility that patients with normal blood pressure or easily controlled hypertension have less severe underlying disease. Subsequent clinical trials have confirmed the benefit of antihypertensive therapy, particularly with ACE inhibitors, in patients with nondiabetic chronic kidney disease. Therefore, hypertension should be aggressively controlled in most patients by virtually any agent(s) shown to be effective. The possible exception is patients with proteinuric kidney disease, for whom ACE inhibitors may be the drug of choice for management of

hypertension. However, the MDRD trial (135) did not address the potential preferential benefit of ACE inhibitors. This issue has now been addressed in trials involving ramipril (138) and benazepril (142). The later study consisted of almost 600 patients with a variety of chronic kidney diseases. The patients were already in reasonable blood pressure control on a variety of different medications and then were randomized to benazepril or placebo. Benazepril produced a greater reduction in blood pressure (3.5–5.0 vs. 0.2 mm Hg reduction in diastolic pressure) than placebo and was associated with a 25% reduction in protein excretion. Progression to a primary end point (defined as doubling of the plasma creatinine concentration or progression to dialysis) occurred in 31 of 300 patients treated with benazepril versus 57 of 283 in the placebo group. The risk reduction was 53% in the entire group, 71% in those with a baseline creatinine clearance >45 mL/min/1.73 m^2, and 46% in those with a baseline creatinine clearance <45 mL/min/1.73 m^2. There was clear benefit in patients with chronic glomerular diseases and diabetic nephropathy. A more recent study suggested that there is a lower rate of loss of GFR in patients with essential hypertension who are treated with an ACE inhibitor compared to a β-blocker despite equivalent degrees of blood pressure control. A benefit also was noted in a report from the Ramipril Efficacy in Nephropathy (REIN) trial (138) in which patients with nondiabetic chronic kidney diseases were randomized to ramipril or placebo plus other antihypertensive therapy to attain a diastolic pressure <90 mm Hg. The trial was terminated prematurely among patients excreting >3 g protein/day because of a significant benefit with ACE inhibition in ameliorating the rate of decline of kidney function (0.53 vs. 0.88 mL/min/mo for placebo). In a follow-up study of those initially enrolled in the ramipril trial (143,144), the benefits with ramipril continued over time among patients excreting >3 g protein/day. The mean rate of decline of the GFR decreased from 0.44 to 0.10 mL/min/1.73 m^2 for patients originally randomized to ramipril, and from 0.81 to 0.14 mL/min/1.73 m^2 for those not originally given ramipril. Benefits with ramipril also appear to extend to those with lesser degrees of proteinuria. However, the original and follow-up ramipril studies strongly suggest that patients who particularly benefit are those with prominent proteinuria, a finding similar to that noted in the MDRD trial. Additional evidence in support of a preferential benefit with ACE inhibitors has come from two meta-analyses with nondiabetic patients (145,146). Strict control of the blood pressure is beneficial in slowing the rate of progression of nondiabetic chronic kidney disease.

ACE inhibitors appear to be more protective than other antihypertensive drugs in chronic glomerular diseases and patients excreting >1 g protein/day. However, hypertensive African Americans' blood pressure responds better to monotherapy with a calcium channel blocker than an ACE inhibitor. Despite this observation, an interim analysis of the African-American Study of Kidney Disease and Hypertension (AASK) (147,148) found that amlodipine was less effective than ramipril in slowing progression of renal disease in African Americans with hypertensive kidney disease. In early ADPKD, rigorous blood pressure control (95/60 to 110/75 mm Hg) when compared with standard blood pressure control (120/70 to 130/80 mm Hg) was associated with a slower increase in total kidney volume, no overall change in the estimated GFR, a greater decline in left ventricular mass index, and greater reduction in urinary albumin excretion. The combination of lisinopril and telmisartan did not significantly alter the rate of increase in total kidney volume in early ADPKD (149). In patients with ADPKD and Stage 3 CKD, monotherapy with an ACE inhibitor was associated with adequate blood pressure control. The addition of an ARB did not alter the decline in the estimated GFR (150). The results of these studies are summarized in Table 11-10.

Overall, the optimal blood pressure in hypertensive patients has not been clearly identified yet. However, the ABCD trial has demonstrated the importance of intensive blood pressure control (124–126). The rate of loss of GFR appears to be more rapid when the mean arterial pressure remains at or >100 mm Hg (which reflects a diastolic pressure of 80 to 85 mm Hg in the absence of systolic hypertension). Thus, a diastolic pressure goal of 80 mm Hg (mean arterial pressure 98 mm Hg in the absence of systolic hypertension) appears reasonable in previously hypertensive patients excreting 1 to 2 g protein/day. A lower diastolic pressure of 75 mm Hg (mean arterial pressure 92 mm Hg) may be appropriate in those patients who are normotensive with heavy proteinuria.

Table 11–10 Antihypertensive Therapy and Progression of Renal Failure

Trial Name	Measures	Results
MDRD, 1994	Aggressive control vs. usual BP control in patients with	The higher the proteinuria (>3, 1–3, and <1 g/d) the faster the progression of nephropathy and more

	chronic renal diseases	effective the BP control became. Blacks may benefit more.
Maschio et al., 1996	Benazepril vs. placebo in patients with chronic renal diseases	Better BP control, less proteinuria, slower progression rate of nephropathy with benazepril, especially in patients with glomerular diseases and diabetic nephropathy. Inconclusive results in hypertensive nephrosclerosis. No observed benefit in ADPKD patients.
REIN, 1997 and follow-up, 1998, 1999	Ramipril vs. placebo in nondiabetic patients with chronic renal diseases	Ramipril reduced the progression of nephropathy in patients with proteinuria >3 g/d. Sufficient treatment (>3 y) eliminated the need for dialysis. Some patients even showed improved GFR.
UKPDS, 1998	Tight BP control (<150/85 mm Hg) vs. moderate control hypertensive in type II diabetes (<180/105 mm Hg)	Tight BP control decreased the incidence of stroke, diabetic-related death, and microvascular complications, including retinopathy and nephropathy.
AASK, 2000	Ramipril vs. amlodipine in African Americans with hypertensive renal diseases	Ramipril was more effective than amlodipine in slowing progression of nephropathy in blacks.
Meta-analysis, 2001	ACE inhibitors in nondiabetic nephropathy patients	Patients with ACEI treatment were less likely to develop end-stage renal disease and have slower rate of progression of renal function.
Parving et al., 2001	Irbesartan vs. placebo in patients with type II diabetic nephropathy and microalbuminuria	Irbesartan slowed the rate of progression to diabetic nephropathy.

Lewis et al., 2001	Irbesartan vs. placebo in patients with nephropathy owing to type II diabetes	Irbesartan protected against the progression of nephrology.
RENAAL, 2001	Losartan vs. placebo in patients with nephrology owing to type II diabetes	Less proteinuria and slower progression of nephropathy with losartan treatment
ABCD, 1998–2002	Hypertensive type II diabetic patients	Myocardial infarctions decreased with ACEI vs. calcium channel blockers. BP control stabilized or decreased the rate of decline in renal function. Intensive vs. moderate BP control decreased all-cause mortality.
ABCD, 1998–2002	Normotensive type II diabetic patients	BP control stabilized or decreased the rate of decline in renal function. Intensive BP vs. moderate control slows progression from normoalbuminuria to microalbuminuria and the progression of diabetic retinopathy and decreased strokes.
HALT-PKD (Study A)	Early ADPKD. Low BP target vs. standard BP target and either ACEI plus an ARB or ACEI plus placebo	A low BP target was associated with a slower increase in total kidney volume, no overall change in the estimated GFR, a greater decline in left ventricular mass index, and greater reduction in urinary albumin excretion.
HALT-PKD (Study B)	ADPKD with Stage 3 CKD. ACEI and placebo or ACEI and an ARB, with the doses adjusted to achieve a BP of 110/70 to 130/80 mm Hg	Monotherapy with an ACEI was associated with adequate BP control. The addition of an ARB did not alter the decline in the estimated GFR.

AASK, African-American Study of Kidney Disease and Hypertension; ABCD, Appropriate Blood Pressure Control in Diabetes trial; ACEI, angiotensin-converting enzyme inhibitor; ADPKD, autosomal dominant polycystic kidney disease; ARB,

angiotensin receptor blocker; BP, blood pressure; CKD, chronic kidney disease; GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; REIN, Ramipril Efficacy in Nephropathy; RENAAL, Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan; UKPDS, UK Prospective Diabetes Study.

Management of the Uremic State

FLUID AND ELECTROLYTES

Because of the accompanying decrease in tubule processing of the filtrate associated with progressive functional loss, a greater fraction of the filtrate is excreted as urine. As a result, the patient with chronic kidney disease is able to maintain fluid and major elemental balance throughout the course of renal functional deterioration until GFR has fallen to a critical value of <5% of normal. By maintaining a urine volume of ˜2 L/day, the patient with advanced kidney disease is able to excrete the normal fluid (2 L), sodium (140 mEq), potassium (70 mEq), osmotic load (600 mOsm), and nitrogen (12 g, equivalent to 72 g protein) present in the average American diet without excreting a concentrated or dilute urine. Therefore, dietary restrictions, which may cause protein–calorie malnutrition, are not required in the majority of uremic patients throughout the entire course of chronic kidney disease up to and including end-stage disease (135). Similarly, dietary sodium and potassium restriction is not required in the majority of patients with chronic kidney disease and actually can be harmful, as described. A high-potassium diet, potassium-sparing diuretics, and high-sodium diet are contraindicated in advanced kidney disease because of hyperkalemia, hypertension, and fluid overload, respectively.

The mild acidosis present in the majority of patients with advanced chronic kidney disease can be treated readily with the administration of 12 mEq (1 g) of sodium bicarbonate given thrice daily. This amount of bicarbonate has minimal effect on blood pressure and ECF volume (151); however, not all nephrologists believe there is a need to treat the mild acidosis of chronic kidney disease.

Chronic kidney disease causes edema by two distinct mechanisms: (a) hypoalbuminemia (serum albumin <2.5 g/dL) secondary to urinary protein loss and (b) when the ingestion of sodium and water exceeds the ability of the diseased kidney to eliminate the ingested loads. In general, development of edema from the latter cause is a consequence of advanced

kidney disease and marked reduction in GFR. This frequently necessitates institution of treatment for ESRD (i.e., dialysis or transplantation) because sodium and water restriction with loop diuretics generally is ineffective.

CALCIUM AND PHOSPHATE

Abnormalities in mineral metabolism are well established in chronic kidney disease. As kidney function declines, calcium levels decrease, and PTH and phosphorus levels increase. Numerous observational studies have shown a significant and independent association between elevated serum phosphorus levels and all-cause and cardiovascular mortality in patients with chronic kidney disease (152). Similar findings have been found in chronic kidney disease patients not requiring dialysis. In a study by Kestenbaum et al. (153) of 6,730 patients with chronic kidney disease, serum phosphorus was significantly and independently associated with mortality. For each 1 mg/dL increase in serum phosphorus, the risk for death increased by 23%. Furthermore, after adjustment, serum phosphorus levels >3.5 mg/dL were associated with a significantly increased risk of death and the risk increased linearly with each subsequent 0.5 mg/dL increase in serum phosphorus levels. The mechanism by which serum phosphorus contributes to cardiovascular disease is unclear. It may involve the development of vascular calcification, since serum phosphorus levels have been associated with the presence and extent of coronary artery and aortic calcification in dialysis patients. Elevated serum phosphorus may also contribute to cardiovascular disease by increasing PTH levels. Increased PTH has been associated with left ventricular hypertrophy and an increased risk of cardiovascular and all-cause mortality in dialysis patients. Hence, serum phosphorus appears to be an important predictor of cardiovascular disease. Successful clinical management of hyperphosphatemia consists of several measures including a low-phosphorus diet, adequate dialysis, and effective phosphate-binding therapy. Phosphorus restriction should begin when the GFR is <60 to 70 mg/minute. However, it is difficult to balance dietary phosphate restrictions against the need for adequate protein intake (154–157). Low serum albumin has been shown to be a frequent cause of morbidity and mortality. Dialysis does not generally provide adequate phosphorus control because it is difficult to remove a significant amount of the total body phosphorus that is in the intracellular compartment. Thus, almost all dialysis patients rely on phosphate binders taken with meals to reduce the absorption of dietary phosphorus and prevent hyperphosphatemia. Those

agents include aluminum-, calcium-, other metal-, and other non–metal-based preparations, all of which have limitations. Although extremely efficient, aluminum-containing binders can cause toxic effects such as aluminum bone disease (osteomalacia), dementia, myopathy, and anemia (99,158,159). This has led to the alternative use of calcium salts. Calcium carbonate and calcium acetate are the most efficacious therapies. Calcium citrate should be avoided because it can increase intestinal absorption of aluminum. Currently, calcium carbonate and calcium acetate are the most widely used phosphate binders (160). To improve their effectiveness and decrease the chance of causing hypercalcemia, these drugs (1–2 g) should be given with meals. However, calcium-containing phosphate binders can cause an excess total body calcium load because of significant intestinal calcium absorption. There has been interest in developing calcium- and aluminum-free binders such as magnesium carbonate. A lower dialysate magnesium concentration may be needed to prevent hypermagnesemia. Metal-free binders have also been developed such as sevelamer (RenaGel) and lanthanum. Sevelamer is a nonabsorbable agent that contains neither calcium nor aluminum. This drug is a cationic polymer that binds phosphate through ion exchange. Several studies have reported that this agent is an effective phosphate binder without affecting plasma calcium. It also has the advantage of lowering total cholesterol concentration. Gastrointestinal side effects may limit its use in some patients. Because of its much higher cost, it is primarily reserved for patients with hypercalcemia (161–163).

ANEMIA

Anemia is common in patients with chronic kidney disease. It is well established that hemodynamic changes induced by anemia can precipitate high-output congestive heart failure and ischemic cardiac events (164,165). If treatment is required before ESRD because of associated conditions such as generalized weakness, cardiac or pulmonary disease, EPO, although quite expensive, is effective (166). Before EPO administration, iron stores should be documented to be adequate by the measurement of serum ferritin, iron, and total iron-binding capacity. The initial dosage of EPO should be 75 to 100 units/kg/wk. An adequate response to EPO therapy will result in an increased reticulocyte count within 1 week and rise in hematocrit after 2 to 4 weeks. A potential complication of increasing the hematocrit with EPO is hypertension, which can have an adverse effect on kidney function.

BLEEDING DIATHESIS

The bleeding diathesis present in uremia usually requires no treatment unless the patient requires surgery or experiences a traumatic event. Correction of anemia with either transfusion of packed cells or administration of recombinant human EPO may improve hemostasis. The minimum target hemoglobin should be ˜10 g/dL. Fifty to seventy-five percent of uremic patients have improvement or correction of the bleeding time with dDAVP. The usual dosage is 0.3 μg/kg given intravenously, subcutaneously, or intranasally (167–169). It is effective within 1 to 2 hours, with its effect persisting for ˜4 hours. Tachyphylaxis can occur within 24 to 48 hours. Conjugated estrogens improve bleeding time in ˜80% of the patients. The usual dosage is 0.6 mg/kg daily for five consecutive days. The initial effect on bleeding time occurs within 6 hours with peak response in 5 to 7 days, which persists for up to 14 days (170–172). Cryoprecipitate also is effective in controlling the uremic bleeding diathesis. The usual infusion of cryoprecipitate is 10 bags. It affects the bleeding time within 1 hour and persists for 18 hours. Platelet infusions also are effective but are recommended only for life-threatening emergencies.

MISCELLANEOUS DISTURBANCES

In general, other disturbances that occur with chronic kidney disease such as mild glucose intolerance, hypertriglyceridemia, and mild elevation of uric acid require no treatment. However, because of the inability of renal excretion of large loads of magnesium, magnesium-containing antacids, and laxatives should be largely avoided. Similarly, as stated previously, potassium supplementation, even with diuretic administration, potassium-sparing diuretics, and salt substitutes, should be avoided unless hypokalemia is present.

Preparation for and Initiation of End-Stage Renal Disease Therapy

Chronic kidney disease progresses at a variable rate because of differences in the clinical course of the underlying diseases and availability of different potential therapeutic interventions. A number of factors have been proposed as possible indicators before development of uremic

symptoms (173–177). It is important to note that plasma creatinine reflects not only kidney function but also muscle mass (178,179). Thus, a patient with reduced muscle mass owing to malnutrition has a lower than usual plasma creatinine level and may need to be dialyzed (180,181). It is difficult to assign a certain level of BUN, serum creatinine, or GFR to the need to start dialysis. Nevertheless, the Health Care Financing Administration in the United States has assigned levels of serum creatinine and creatinine clearance to quantify for reimbursement from Medicare for patients receiving dialysis. Serum creatinine must be ≥8.0 mg/dL and the GFR must be ≤10 to 20 mL/min/1.73 m^2. Symptomatic uremia usually develops when serum creatinine reaches 8 to 10 mg/dL and BUN is >100 mg/dL. Classically, the first manifestations are gastrointestinal symptoms of nausea, anorexia, and possibly vomiting. However, other symptoms and findings of advanced uremia, such as intractable itching, malnutrition, volume overload, protracted hyperkalemia, and impairment of cognitive function represent additional indications for considering ESRD treatment. Serositis (pericarditis and pleuritis) does not respond to conservative measures but usually resolves following initiation of dialysis or transplantation. In addition, uremic motor neuropathy is a progressive and debilitating condition in the uremic state. Its progression can be prevented by adequate dialysis or transplantation.

Data from the U.S. Renal Data System have shown that the majority of patients who began renal replacement therapy, either by dialysis or transplantation, have advanced complications of uremia such as malnutrition, severe anemia, left ventricular hypertrophy, or congestive heart failure (182,183). This suggests the drawbacks of delaying renal replacement therapy for ESRD and possibly inadequate care during the pre-ESRD period (184,185). The processes contributing to cardiovascular disease begin early during the period of kidney disease. Some factors such as hypertension, dyslipidemia, anemia, and hyperparathyroidism are amenable to early intervention.

Consequently, patients with chronic kidney disease should be referred to nephrologists early in the course of their disease. Furthermore, it is important to counsel patients in preparation for optimal renal replacement therapy and to prevent the morbidity and mortality associated with chronic kidney disease. Current clinical practice guidelines (Dialysis Outcomes Quality Initiative Guidelines, NKF-DOQI) in the United States and Canada recommend earlier initiation of dialysis before the overt symptoms and signs of uremia.

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CHAPTER 12

Obstructive Nephropathy: Pathophysiology and Management

Kevin P. G. Harris

Introduction

Urine that is produced by the kidney is conveyed to the urinary bladder by the renal pelvis and ureter. This relies on peristalsis occurring within the ureters and, during high urine flow rates, the pressure gradient along them. The urinary bladder is a muscular and distensible storage compartment with a capacity of up to 800 mL in humans. At about a capacity of 150 to 400 mL, stretch receptors result in neurologic signals which relax the involuntary internal urethral sphincter and result in the sensation of needing to urinate, although urination may be delayed by conscious control of the external urethral sphincter as long as the capacity of the bladder is not exceeded. Conscious relaxation of the external urethral sphincter allows urine to be voided through the urethra.

The normal elimination of urine from the body may be affected by pathology anywhere along the urinary tract which either results in a physical barrier to urine flow or disrupts the complex neurologic processes which control it. This is referred to as *obstructive uropathy*. Typically

dilatation of the urinary tract or *hydronephrosis* then occurs proximal to the site of obstruction, a change which can be readily detected with a variety of imaging techniques. However, hydronephrosis is not synonymous with obstructive uropathy as it can occur without functional obstruction to the urinary tract and can be absent in established obstruction, for example in vesicoureteral reflux (VUR), primary megaureter, and diabetes insipidus.

Impedance to the flow of urine initially results in a high back pressure which causes a number of direct and indirect functional effects on the renal parenchyma, referred to as *obstructive nephropathy*.

Immediately following acute urinary tract obstruction, changes within the kidney are mainly functional resulting in acute kidney injury (AKI) which may recover with prompt and effective relief of the obstruction. If left untreated, obstruction will result in irreversible structural damage and scarring within the kidney leading to chronic kidney disease (CKD). The management of obstruction to the urinary tract requires close collaboration between nephrologists and urologists in order to minimize long-term and irreversible damage to the kidney, but despite this urinary tract obstruction remains a major cause of CKD worldwide.

Obstructive uropathy is classified as to whether it is acute (less than a few days duration) or chronic and whether it is complete (high grade) or incomplete and partial (low grade). Obstruction is further subdivided into whether it affects the upper urinary tract obstruction (usually unilateral obstruction occurring above the vesicoureteral junction [VUJ]) or lower urinary tract obstruction (usually bilateral obstruction located below the VUJ). Classifying obstruction in this way predicts the likely pathophysiologic effects on the patient; for example, unilateral obstruction in a patient with two normal kidneys will not result in significant renal impairment because the contralateral kidney compensates, but bilateral obstruction or the obstruction of a single functioning kidney will result in renal failure.

Obstruction may result from either acquired or congenital abnormalities. Acquired urinary tract obstruction may affect either the upper or lower urinary tract and can result from either intrinsic or extrinsic causes. Intrinsic causes of obstruction may be intraluminal or intramural.

With increased use and improved sensitivity of antenatal scanning, congenital abnormalities of the urinary tract are now frequently identified early, allowing prompt postnatal (and in some cases antenatal) intervention to relieve the obstruction and hence preserve renal function (1). If obstruction occurs early during development, the kidney fails to develop

and becomes dysplastic. If the obstruction is bilateral, there is a high mortality rate as a result of severe renal failure. If the obstruction occurs later in gestation and is low grade or unilateral, hydronephrosis and nephron loss will still occur but renal function may be sufficient to allow survival. Such patients may not present until later in life or only be discovered as an incidental finding.

Incidence and Prevalence

Obstructive uropathy is a common entity and can occur at all ages. The exact incidence of obstructive uropathy is difficult to ascertain, since obstruction occurs in a variety of diseases that may warrant hospitalization and surgical intervention and may be transient. However, the prevalence of hydronephrosis at autopsy is 3.5% to 3.8% of adults and 2% of children, with about equal distribution between males and females (2).

The frequency and etiology of obstruction vary in both sexes with age. Congenital urinary tract obstruction occurs most frequently in males, commonly as a result of either posterior urethral valves or pelvic-ureteral junction (PUJ) obstruction explaining the higher rate of obstructive uropathy in male children less than 10 years old.

In the United States, congenital obstructive uropathy remains the single most common cause of end-stage renal disease (ESRD) in pediatric patients (0–21 years) accounting for about 9% of incident patients (3) and despite improvements in the treatment, this condition may continue to impact into adult life.

Beyond 20 years of age, obstruction becomes more common in females, mainly as a result of pregnancy and gynecologic malignancies. Urolithiasis occurs predominantly in young adults (25–45 years old) and is three times more common in men than in women. In patients older than 60 years, obstructive uropathy is seen more frequently in men, secondary to benign prostatic hyperplasia and prostatic carcinoma. About 80% of men older than 60 years have some symptoms of bladder outflow obstruction, and up to 10% have hydronephrosis. Although the exact relationship between symptoms of bladder outflow obstruction and CKD is unclear, there appears to be a significant association between a decreased peak flow rate and CKD (4).

In the United States the incidence of ESRD due to acquired obstruction is 0.9% with 76% being male and 61% being over the age of 65 (3). However, this is dwarfed by comparison with other causes of ESRD in the

adult population such as glomerular disease, diabetes, and hypertension.

Causes of Obstructive Uropathy

The major causes of obstructive uropathy are described in Table 12-1.

Intrarenal tubular obstruction can result from the deposition of uric acid crystals in the tubular lumen after treatment of hematologic malignancies (tumor lysis syndrome), with the precipitation of Bence Jones protein in myeloma and with the precipitation or crystal formation of a number of drugs, including sulfonamides, acyclovir, methotrexate, and indinavir.

Renal calculi, which typically lodge in the calyx, PUJ, or VUJ and at the level of the pelvic brim are the most common cause of extrarenal intraluminal obstruction. Calcium oxalate stones (the most common form) typically cause intermittent acute unilateral urinary tract obstruction in young adults, but rarely significant renal impairment. Struvite, urate, and cystine stones are more often bilateral and more likely to cause long-term renal impairment. Papillary necrosis and a sloughed papilla from diabetes mellitus, sickle cell trait or disease, analgesic nephropathy, renal amyloidosis, and acute pyelonephritis may result in intraluminal obstruction as may blood clots following macroscopic hematuria as a result of renal tumors, arteriovenous malformations, renal trauma, or in patients with polycystic kidney disease (clot colic).

Intramural obstruction can result from either functional or anatomic changes. Functional disorders include VUR, adynamic ureteral segments (usually at the junction of the ureter with the pelvis or bladder), and neurologic disorders. The latter may result in a contracted (hypertonic) bladder or a flaccid (atonic) bladder, depending on whether the lesion affects upper or lower motor neurons, and lead to impaired bladder emptying with VUR. Bladder dysfunction is very common in patients with multiple sclerosis and after spinal cord injury, and is also seen in diabetes mellitus and Parkinson disease and after cerebrovascular accidents. Some drugs (anticholinergics, levodopa) can alter neuromuscular activity of the bladder and result in functional obstruction, especially if there is preexisting bladder outflow obstruction (e.g., prostatic hypertrophy). Anatomic causes of intramural obstruction of the upper urinary tract include transitional cell carcinoma of the renal pelvis and ureter and ureteral strictures secondary to radiotherapy or retroperitoneal surgery. Rarely, obstruction may result from ureteral valve malfunction, polyps, or

strictures after therapy for tuberculosis. Intramural obstruction of the lower urinary tract can result from urethral strictures, which are usually secondary to chronic instrumentation or previous urethritis, or malignant and benign tumors of the bladder. Infection with *Schistosoma haematobium*, when the ova lodge in the distal ureter and bladder, is a common cause of obstructive uropathy worldwide, with up to 50% of chronically infected patients developing ureteral strictures and fibrosis with contraction of the bladder.

Table 12–1 Causes of Obstruction in the Urinary Tract

Upper Urinary Tract	Lower Urinary Tract
Intrinsic Causes Intraluminal • Intratubular deposition of crystals (uric acid, drugs) or Bence Jones protein • Ureter: stones, clots, renal papillae, fungus ball Intramural • Ureteropelvic or ureterovesical junction dysfunction • Ureteral valve, polyp, stricture, or tumor	**Anatomic Causes** • Phimosis, meatal stenosis, paraphimosis • Urethra: strictures, stones, diverticulum, posterior anterior urethral valves, urethral infections, periurethral abscess, uretheral surgery • Prostate: benign prostatic hyperplasia, abscess, prostatic carcinoma • Bladder: calculus, malignancy • Trauma, straddle injury • Pelvic fracture
Extrinsic Causes Originating in the reproductive system • Uterus: pregnancy, prolapse, tumors, endometriosis • Ovary: abscess, tumors, • Cervix: carcinoma • Prostate: carcinoma Vascular system • Aneurysm: abdominal aorta, iliac vessels • Aberrant vessels: ureteropelvic junction • Venous: retrocaval ureter, ovarian vein • Fibrosis following vascular	**Functional Causes**

reconstructive surgery

Lesions of the gastrointestinal tract
- Crohn disease
- Diverticulitis
- Appendiceal abscess
- Tumors, abscess, cyst

Diseases of the retroperitoneum
- Retroperitoneal fibrosis (idiopathic radiation)
- Inflammatory: tuberculosis, sarcoidosis
- Hematomas
- Primary retroperitoneal tumors (lymphoma, sarcoid)
- Metastatic disease in the retroperitoneum (cervix, bladder, colon, prostate)
- Lymphocele

Inadvertent surgical ureteric ligation

Neurogenic bladder: spinal cord
- defect or trauma, diabetes, multiple sclerosis, cerbrovascular accidents, Parkinson disease
- Drugs: anticholinergics, antidepressants, L-DOPA

While ureteral dilation without functional obstruction is commonly seen in pregnancy as a result of hormonal effects (especially progesterone) on smooth muscle, extrinsic obstruction sometimes resulting from compression of the urinary tract is pressure from a gravid uterus on the pelvic rim with the right ureter being more commonly affected. It is usually asymptomatic, the changes resolve rapidly after delivery, and AKI from bilateral obstruction is very rare.

Extrinsic obstruction may result from malignancy compressing or directly invading the urinary tract. Direct extension of the tumor to involve the urinary tract occurs in up to 30% of patients with carcinoma of the cervix. Other pelvic pathologies that can cause ureteral compression include benign and malignant uterine and ovarian masses, abscesses, endometriosis, and pelvic inflammatory disease.

In males, the most common cause of extrinsic obstruction of the lower urinary tract is benign prostatic hypertrophy. Carcinoma of the prostate can also result in obstruction either from direct tumor extension to the bladder outlet or ureters or from metastases to the ureter or lymph nodes.

Retroperitoneal pathology such as primary or secondary tumors or inflammatory disease may also result in extrinsic obstruction of the ureters. Retroperitoneal fibrosis, in which a thick fibrous tissue extends out from the aorta to encase the ureters and draw them medially, may be

idiopathic or result from inflammatory aortic aneurysms, certain drugs (e.g., β-blockers, bromocriptine, and methysergide), previous radiation, trauma or surgery, and granulomatous disease.

Inadvertent ureteric ligation is a rare but recognized complication of pelvic surgical procedures and may go unrecognized.

The Effects of Urinary Tract Obstruction on Renal Function

The increased pressure that occurs after the onset of ureteral obstruction triggers profound functional and structural changes within the kidney. This increase in pressure is greatest immediately after the onset of obstruction and tends to fall with time with incomplete obstruction. Damage in the obstructed kidney is potentiated by those conditions that acutely increase ureteral pressure, such as increases in urine flow (i.e., an increase in fluid intake or after administration of diuretics) or augmentation of the degree of obstruction or both.

It is rarely possible to accurately define the time of onset of obstruction in humans or to obtain repetitive measurements of renal function. Therefore, our understanding of the consequences of urinary tract obstruction stems mainly from the study of animal models (5). The majority of studies have used invasive techniques to examine the effects of complete short-term ureteral obstruction in rodents. Investigators have also examined models of chronic complete, partial, or reversible obstruction in adult and neonatal animals and modern imaging techniques have been used to noninvasively examine the effects of obstruction on glomerular and tubular function (6). In general, there appears to be little species-to-species variation in the response to acute obstruction, suggesting similar changes are likely to occur in humans. Although initially the changes are predominantly functional and potentially reversible, chronic obstruction results in irreversible structural changes (7), and models of obstruction are often used to examine the pathogenetic mechanisms underlying the development of renal fibrosis from any cause (8).

THE EFFECTS OF ACUTE URETERAL OBSTRUCTION ON GLOMERULAR FUNCTION

Experimental evidence suggests that ureteral obstruction may reduce

glomerular filtration rate (GFR) though effects on (a) the mean difference in hydrostatic pressure between the glomerular capillary lumen and Bowman's space (ΔP); (b) renal plasma flow (Q_A); (c) the ultrafiltration coefficient of the glomerular capillary wall (K_f), which reflects both the total surface area available for filtration and the intrinsic permeability characteristics of the filtering apparatus. The manner in which these parameters are affected depends on the duration of the obstruction, the volume status of the animal, and whether or not a contralateral functioning kidney is present.

GFR falls progressively following the onset of complete ureteral obstruction (9) but can be maintained to some extent by continuous reabsorption of salt and water along the nephron, the ability of the renal tract to dilate, and alterations in renal hemodynamics.

Changes in Hydrostatic Pressure Gradients

Ligation of the ureter increases ureteral pressure causing an immediate increase in proximal tubular pressure, the latter being higher than that in the ureter. The rise in intratubular pressure depends on the degree of hydration of the animal, mean urine flow rate, and whether one or both kidneys are obstructed. Nevertheless, independent of the volume status, intratubular pressure rises within an hour of ureteral obstruction (Fig. 12-1). Concomitantly, there is an increase in glomerular capillary hydrostatic pressure; however, this increase in intraglomerular pressure is not proportional to the rise in intratubular pressure (10). Therefore, the net hydrostatic pressure difference across glomerular capillaries decreases. This results in a decline in GFR. After approximately 5 to 6 hours of ureteral obstruction, proximal intratubular pressure begins to decline (11). After 24 hours, intratubular pressures are lower than (11,12) or equal to (13) values before obstruction in animals with unilateral ureteral obstruction (UUO), but this does not restore an effective filtration pressure, because intraglomerular capillary hydrostatic pressure declines at an even faster rate (11,12) and falls below the levels seen before obstruction. In animals with bilateral ureteral obstruction, proximal intratubular pressures are initially twofold higher (11,14) than those seen in rats with UUO (Fig. 12-1). By 24 hours, the levels of intratubular pressure have fallen but not back to the baseline (14,15). At this time, glomerular capillary pressure is no different from preobstruction values. Thus, in this setting, high intratubular pressures contribute significantly to the decrease in GFR.

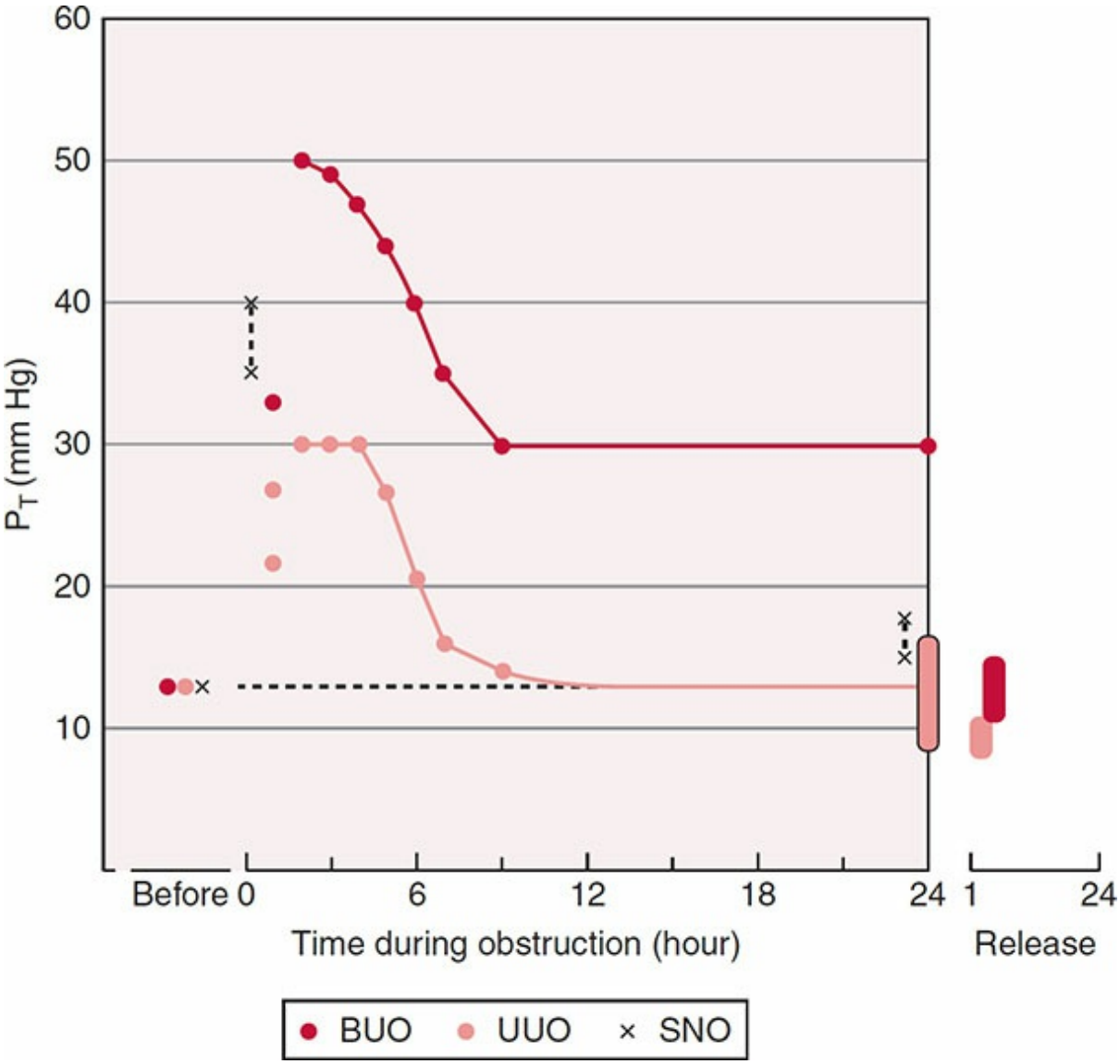

Figure 12–1 Pressure in proximal renal tubules (PT) before, during, and after release of complete obstruction of one ureter (UUO), both ureters (BUO), or single nephrons (SNO). BUO, bilateral ureteral obstruction; SNO, single-nephron obstruction; UUO, unilateral ureteral obstruction.

Changes in Renal Blood Flow

Ureteral obstruction causes a transient increase in renal blood flow (16). Decreased resistance of the afferent arteriole accounts for the increase in blood flow to the unilaterally obstructed kidney (16,17). This phenomenon is observed in both the denervated and the isolated perfused kidney, suggesting that this hyperemic phase is mediated through an intrarenal mechanism. Measurements of the distribution of blood flow during this phase indicate that inner cortical blood flow is increased (18–20). There is a progressive decrease in blood flow to the inner medulla during ureteral

obstruction (21). This increase in renal blood flow may represent a hemodynamic response intended to maintain GFR. The increase in renal blood flow and afferent arteriolar dilatation leads to an increase in glomerular capillary pressure. This response maintains GFR at approximately 80% of preobstruction values despite the substantial increase in proximal tubular pressure. The mechanism underlying this response involves a signal generated at the single-nephron level because a wax plug placed in the proximal tubule generates an identical hemodynamic response in a single glomerulus. Tanner (22) suggested that the fall in afferent arteriolar resistance was caused by tubular-glomerular feedback related to interrupting acutely distal delivery of tubular fluid to the macula densa. Ichikawa (23), however, demonstrated that glomerular blood flow does not rise if proximal tubular pressure is maintained in the normal range in the face of tubule blockade, suggesting that the altered glomerular hemodynamics are a result of intratubular dynamics rather than cessation of distal delivery of tubule fluid. The transient increase in renal blood flow after ureteral obstruction can be prevented by the administration of inhibitors of prostaglandin synthesis such as indomethacin (24). Thus, vasodilator prostaglandins, such as prostaglandin E_2 and prostacyclin, may account for this initial vasodilator effect. At this time interval, the renal vascular bed is particularly resistant to vasoconstriction induced by either electrical stimulation of renal nerves or an infusion of catecholamines. In addition, autoregulation of renal blood flow is impaired, suggesting a prominent vasodilating influence following the onset of ureteral obstruction. Usually the increase in blood flow following obstruction peaks at about 2 to 3 hours.

In a second phase, approximately 3 to 5 hours after the onset of obstruction, renal blood flow starts to decline, while ureteral pressure continues to increase. In part, this may be a consequence of augmented renal resistance owing to increased interstitial pressure. In this phase, ureteral pressure starts to fall toward control values, and renal plasma flow continues to decline, reaching about 30% to 50% of control values by 24 hours (25,26). This vasoconstrictive response of the kidney to UUO results predominantly from an increased resistance of afferent arterioles.

In animals with bilateral ureteral obstruction, the changes in renal hemodynamics are similar to those seen following UUO. There also is an initial hyperemic phase (14,16) that is blocked by cyclooxygenase inhibitors (24), and the decline in GFR thus is secondary to a rise in intratubular pressure. Renal plasma flow falls progressively and is similar at 24 hours to that seen after UUO, although afferent arteriole resistance

may not increase as much. As a result of the persistently high proximal tubular pressure and decline in renal plasma flow, it would be expected that the decline in GFR would be greater after bilateral ureteral obstruction than after UUO. However, this is not the case and may reflect the effect of a higher intraglomerular capillary pressure and greater number of filtering nephrons before and after release of obstruction of 24 hours' duration in rats with bilateral ureteral obstruction than in those with UUO (27).

Changes in the Ultrafiltration Coefficient

After ureteral obstruction, GFR falls to a greater extent than renal plasma flow (9). Thus, the filtration fraction decreases. This may reflect preferential constriction of the preglomerular blood vessels because this would lower both blood flow and glomerular capillary pressure, thus resulting in a greater decrement in GFR than in blood flow. Alternatively, it is suggested that there is either diversion of blood to nonfiltering areas of the kidney or a reduced area available for filtration per glomerulus. That the latter occurs is suggested by the finding that K_f values in rats with ureteral obstruction are lower than those typically obtained in normal rats (28).

In summary, the fall in single-nephron GFR in obstruction is caused by a decrease in net hydrostatic pressure across the glomerular capillary wall. The fall in net hydrostatic filtration pressure initially is caused by an increase in intratubular pressure. After 24 hours of obstruction, the main mechanism responsible for the decrement in net hydrostatic pressure across the glomerular capillary wall is a fall in intraglomerular pressure. In animals with bilateral ureteral obstruction, both a persistent increase in intratubular pressure and a decrease in intraglomerular pressure contribute to the decrease in net hydrostatic pressure across glomerular capillaries. There also is evidence that K_f is decreased. The greater decrease in total kidney GFR than in single-nephron GFR after 24 hours of obstruction results from the fact that some nephrons cease to function during the period of obstruction.

THE EFFECTS OF PROLONGED URETERAL OBSTRUCTION ON GLOMERULAR FUNCTION

After complete ureteral obstruction in the rat, GFR reaches 2% of control values by 48 hours and remains at this low level. Renal plasma flow also declines but to a lesser extent (25). The effects of partial chronic

obstruction of the ureter depend on both the degree and the duration of the obstruction. Whole-kidney GFR may be reduced to one-third of control values 2 to 4 weeks following partial ureteral obstruction in the rat (29). Single-nephron GFR, however, is reduced by only 20% of control levels, suggesting that the decline in whole-kidney function is a result of a loss in the number of functioning nephrons not accessible to micropuncture, that is, juxtamedullary nephrons (30).

Rats with partial obstruction of 2 to 4 weeks' duration have a 30% decrease in K_f. GFR and single-nephron plasma flow are maintained near normal because of an increase in glomerular capillary pressure secondary to a greater decrease in afferent than efferent arteriolar resistance. This vasodilatation is prostaglandin mediated, and indomethacin administration increases both afferent and efferent arteriolar resistance and causes a decline in single-nephron GFR (31).

MODULATORS OF GLOMERULAR FUNCTION FOLLOWING OBSTRUCTION

Experimental studies suggest that the vasoconstrictors angiotensin II and thromboxane A_2 play a central role in the changes in plasma flow per nephron and single-nephron GFR seen after obstruction. Inhibition of thromboxane A_2 synthesis in rats with ureteral obstruction increases plasma flow per nephron, owing to decreased vasoconstriction of both afferent and efferent arterioles (29). Thromboxane also may decrease K_f through mesangial cell contraction and a decrease in the surface area available for filtration. Although infusion of angiotensin II into normal animals increases net filtration pressure, presumably because of greater vasoconstriction of the efferent than the afferent arteriole, blockade of angiotensin II formation after relief of obstruction increases GFR (29). This increase in GFR may result from a greater filtering surface area, because angiotensin II causes mesangial cell contraction and therefore can reduce the total glomerular capillary area available for filtration. In addition, angiotensin II decreases plasma flow per nephron, which also contributes to the fall in single-nephron GFR. The central and critical role of these two vasoconstrictors in modulating postobstructive renal hemodynamics is illustrated by the fact that rats pretreated with both angiotensin-converting enzyme (ACE) and thromboxane synthase inhibitors, before obstruction, demonstrate almost normal renal function after release of obstruction (32).

Vasodilator prostaglandins, produced in increased amounts by the obstructed kidney, may prevent further decrements in GFR by antagonizing the vasoconstrictive effects of thromboxane A_2 and angiotensin II. Indeed, it has been demonstrated that after release of obstruction in rats, in the setting of prior inhibition of the thromboxane synthase, administration of inhibitors of the cyclooxygenase causes a marked decrease in whole-kidney GFR and renal plasma flow (31).

Atrial natriuretic peptide (ANP), which can cause preglomerular vasodilatation and postglomerular vasoconstriction and increase K_f, are higher in rats with bilateral ureteral obstruction than in rats with UUO (33). ANP antagonizes the vasoconstrictive effects of angiotensin II, raising the possibility that the elevated levels of endogenous ANP in animals with bilateral ureteral obstruction minimize the renal vasoconstriction that occurs compared with animals with UUO.

An interstitial leukocyte infiltrate, predominantly macrophages, is an early event following ureteral obstruction. This begins to increase as early as 4 to 12 hours after ureteral obstruction and continues to increase over the course of days thereafter. By 4 days after left ureteral ligation, there is a 20-fold increment in the renal cortical macrophage number in the obstructed kidney versus either the contralateral unobstructed kidney or normal kidneys from age-matched, sham-operated animals (34). The signal for renal leukocyte recruitment immediately after ureteral obstruction is predominantly macrophage specific and appears to plays a key role in the acute functional changes after ureteral obstruction (35).

RECOVERY OF GLOMERULAR FUNCTION AFTER RELEASE OF URETERAL OBSTRUCTION

The degree of recovery of GFR after release of ureteral obstruction depends on the severity and duration of the obstruction. After release of a 2-week complete ureteral obstruction in the dog, GFR in the postobstructed kidney averages 25% of ipsilateral control values and 16% of concurrent values for the contralateral kidney, the latter having undergone a compensatory increase in GFR (36). Subsequently, the GFR of the postobstructed kidney increases, and the GFR of the normal kidney falls, stabilizing at about 2 months after the release of obstruction. However, GFR does not return to normal in the postobstructed kidney, remaining approximately 50% below the value obtained for the contralateral kidney at 2 years. The changes in effective renal plasma flow mirror the changes seen in GFR.

In rats, a permanent decrease in GFR occurs if ureteral obstruction has been present for >72 hours. After obstruction lasting <30 hours, recovery of whole-kidney GFR is complete, although the normalization in GFR may not be a consequence of homogeneous recovery in single-nephron GFR for all nephrons (37). When single-nephron GFR and the number of filtering nephrons are determined using a modification of Hansen's technique, only 85% of the nephrons filter in the postobstructed kidney (37), suggesting the normalization of whole-kidney GFR occurs at the expense of hyperfiltration (increase in single-nephron GFR) in the remaining functional nephrons (Fig. 12-2). There appears to be a permanent decrement in the total number of functional nephrons.

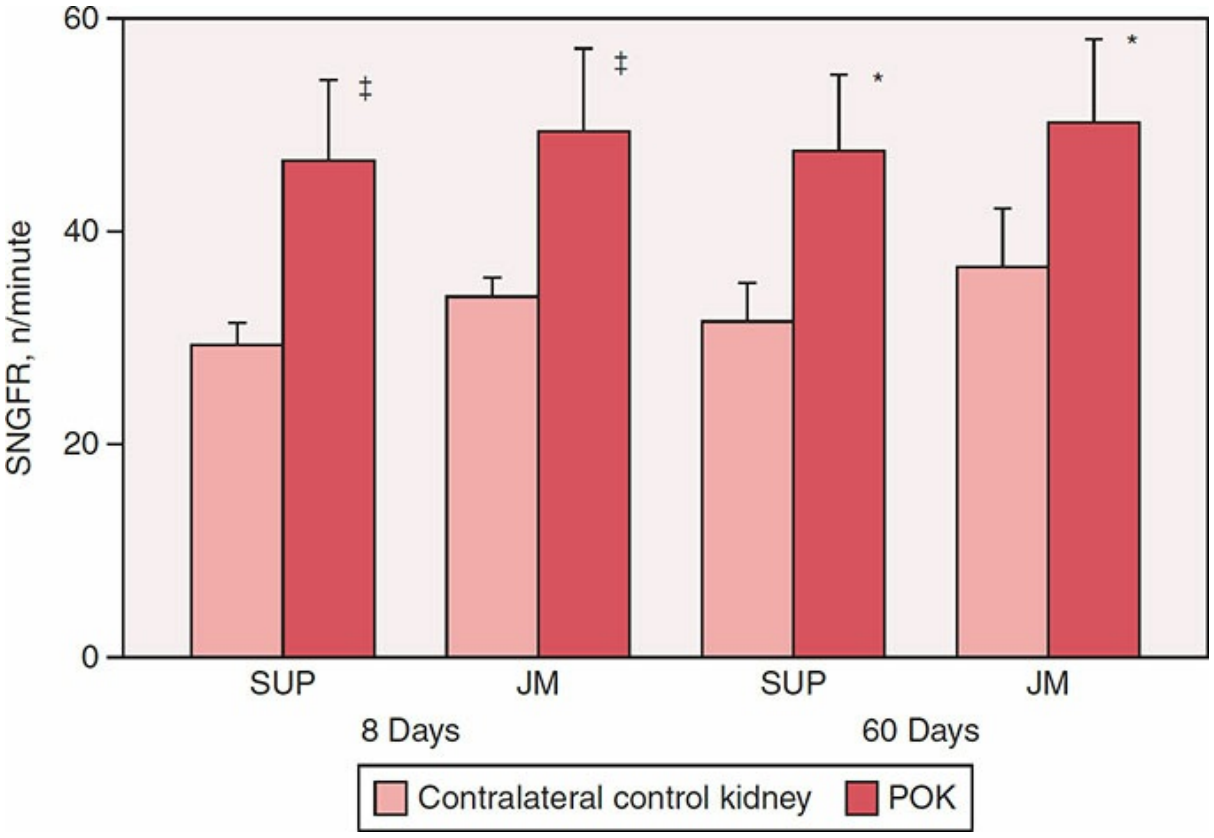

Figure 12–2 SNGFR in SUP and JM nephrons of rats 8 and 60 days after release of UUO of 24 hours' duration. The SNGFR values for the POK were significantly greater (*asterisk*) than those of the contralateral kidney. JM, juxtamedullary; POK, postobstructed kidney; SNGFR, single-nephron glomerular filtration rate; SUP, superficial; UUO, unilateral ureteral obstruction.

The permanent loss of nephrons is likely to be a consequence of fibrosis resulting from renal ischemia and the infiltration into the kidney of biologically active macrophages. The long-term significance of this on the development of significant CKD in adults is unclear particularly if the period of obstruction has been short. However, obstruction to the

developing kidney either prenatally or in childhood appears to have important effects on renal function later in adult life even with effective relief of the obstruction (38).

THE EFFECTS OF URETERAL OBSTRUCTION ON TUBULAR FUNCTION

Urinary tract obstruction results in altered renal handling of electrolytes and changes in the regulation of water excretion with a decreased ability to concentrate the urine. The degree and nature of the tubular defects after obstruction depend in part on whether the obstruction is bilateral or unilateral as a result of the dissimilar hemodynamic responses, different intrinsic changes within the nephron, and differences in extrinsic factors (e.g., volume expansion and accumulation of natriuretic substances in bilateral obstruction).

Sodium and Water Handling

In spite of a decrease in GFR and hence in the filtered load of sodium, the excretion of sodium by the postobstructed kidney of rats with UUO is similar to that of the contralateral kidney (39). Thus, fractional sodium excretion is greater from the postobstructed than from the contralateral kidney. Similar findings have been reported in the dog and in humans after more prolonged periods of obstruction. These findings indicate significant changes in the tubular reabsorption of sodium and water by the previously obstructed kidney. Changes in intravascular volume may affect the absolute and fractional excretion of salt and water by the postobstructed kidney. Absolute sodium excretion after release of UUO is reduced in rats with volume depletion studied under anesthesia when compared with awake rats. In contrast, expansion of the extracellular fluid (ECF) volume with saline solution increases both absolute and fractional sodium excretion. These increases are greater in the postreleased kidney than in the contralateral untouched kidney.

The release of bilateral ureteral obstruction results in a different quantitative excretion of salt and water than what occurs after release of UUO. There is a dramatic increase in the absolute amount of sodium and water excreted in the urine after release of bilateral ureteral obstruction in humans (40) and experimental animals (41,42), resulting in the so-called postobstructive diuresis. The differences in salt and water excretion after release of bilateral ureteral obstruction and UUO may result from

accumulation of osmolytes such as urea and the expansion of the ECF volume during the period of bilateral ureteral obstruction. In addition, the circulating levels of ANP are significantly greater in rats with bilateral ureteral obstruction than in those with unilateral obstruction (33).

Urinary Concentration

Patients with partial obstruction of the urinary tract or patients after relief of partial or complete urinary obstruction have impaired renal concentrating capacity (43), which may take some months to recover following the release of obstruction.

After relief of unilateral obstruction of 24 hours' duration in rats, the urine osmolality from the postobstructed kidney seldom exceeds 400 mOsm/kg H_2O compared with approximately 2,000 mOsm/kg H_2O in the contralateral untouched rat kidney.

The urinary concentrating defect may be explained by both a decreased hypertonicity of the medullary interstitium and a failure of the cortical collecting duct to respond to the action of antidiuretic hormone (ADH). The later may result from a decrease in expression of aquaporin-2 following obstruction to the urinary tract (44).

Obstruction results in a permanent decrease in the number of juxtamedullary nephrons (37). As these have the longest loops of Henle and are responsible for the reabsorption of solutes and the creation of a hypertonic medulla, their loss causes a permanent defect in the concentrating ability of the postobstructed kidney, although this is not as marked as that seen in the acute stages of obstruction.

In addition to the mechanisms described above, following release of bilateral ureteral obstruction, the osmotic effect of solutes retained during the period of obstruction contributes to the generation of isotonic urine after relief of bilateral ureteral obstruction.

Urinary Acidification

In humans (43) and experimental animals (45,46), acid excretion is impaired after the release of obstruction, and returns to normal after some time (months). Studies in experimental animal models of urinary tract obstruction (46) as well as in patients (43) suggest there is a form of distal renal tubular acidosis with an inability to lower the urine pH to normal minimum values in response to acidemia or acid loading.

Potassium Excretion

At any given level of GFR, the fractional excretion of potassium is less in patients with obstructive uropathy than in a comparable group of patients with renal insufficiency caused by a variety of renal diseases (Fig. 12-3). There is a hyperkalemic hyperchloremic acidosis (47) which may be explained at least in part by (a) a deficiency of aldosterone secretion probably secondary to diminished production of renin by the kidney (hyporeninemic hypoaldosteronism), (b) a defect in renal hydrogen ion secretion with an inability to lower pH of the urine maximally in the presence of systemic acidosis and decreased urinary excretion of both ammonium and titratable acid (type 4 distal renal tubular acidosis), (c) a combination of these two defects, or (d) a decreased sensitivity of the distal tubule to the action of aldosterone on potassium secretion.

Excretion of Divalent Cations and Phosphate

Experimental studies have demonstrated a number of changes to the way the kidney handles divalent cations and phosphate following obstruction (48). Fractional excretion of calcium is decreased after release of unilateral obstruction but magnesium excretion increases following release of bilateral or UUO and may result in profound hypomagnesemia.

The reabsorption of phosphate by the postobstructed kidney depends on both the duration of the obstruction and whether the obstruction is bilateral or unilateral. After release of UUO, altered phosphate excretion results primarily from altered renal hemodynamics, and following release of bilateral ureteral obstruction, phosphate excretion is modulated to a large extent by extrarenal factors, mainly the serum levels of phosphate. The obstructed kidney can still respond to exogenous parathyroid hormone administration with an increase in urine phosphate and cyclic 3′5′-adenosine monophosphate excretion, but the magnitude of the response is less in the postobstructed kidney than in the contralateral kidney.

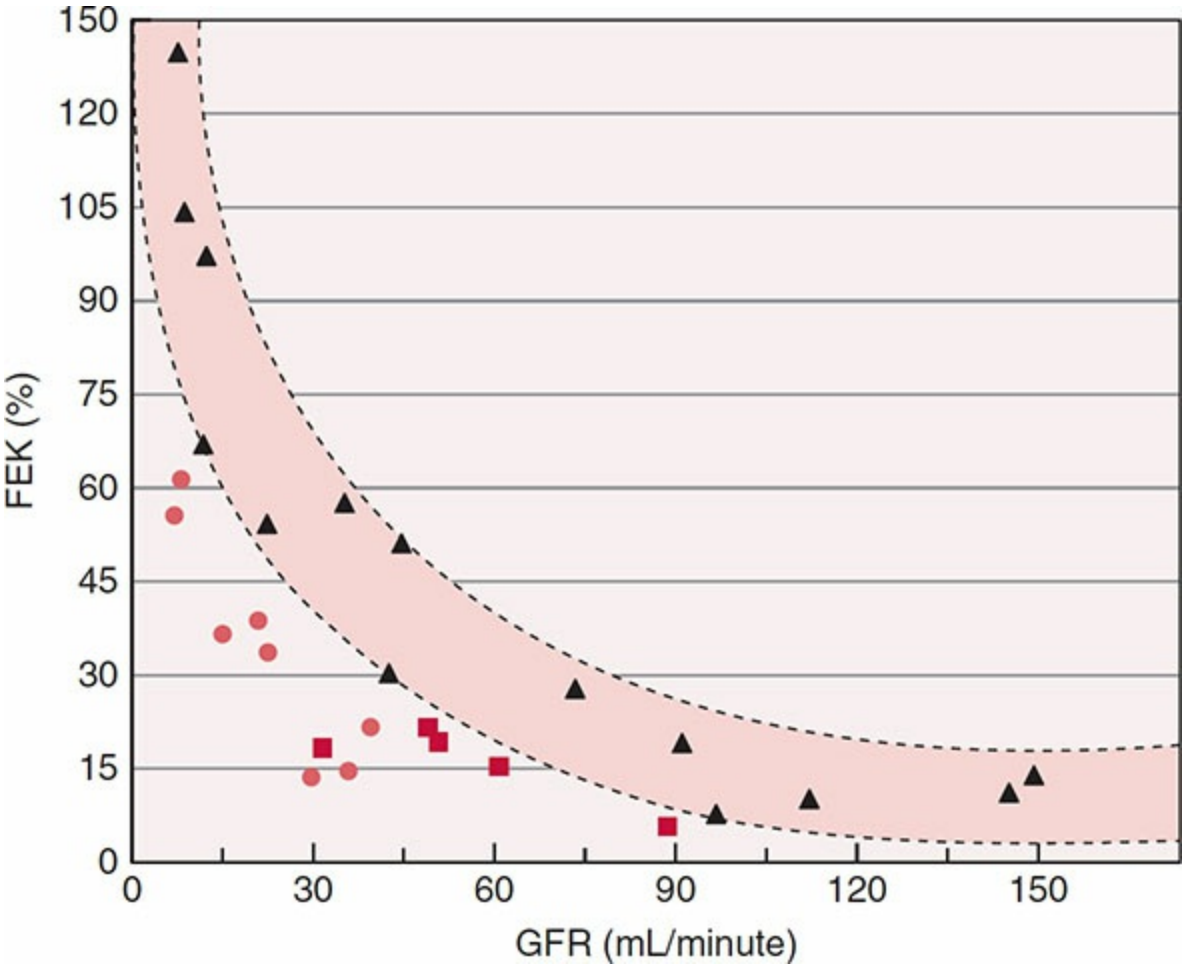

Figure 12–3 Relation of FEK to GFR under baseline conditions. The area inside the broken line depicts the normal adaptive increase in fractional potassium excretion observed with a chronic reduction in GFR. These data were obtained from 14 normokalemic controls (*triangles*) with different GFRs. Each patient (*circle and square symbols*) had a baseline FEK lower than that expected for the corresponding GFR. *Circles* denote patients with distal renal tubular acidosis (*group I*); *open squares* represent patients with hyperkalemic metabolic acidosis owing to selective aldosterone deficiencies (*group II*). FEK, fractional excretion of potassium; GFR, glomerular filtration rate. (From Batlle DC, Arruda JAL, Kurtzman NA. Hyperkalemic distal renal tubular acidosis associated with obstructive uropathy. *N Engl J Med*. 1981;304(7):373–380, Copyright ¿ 2017 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.)

The Effects of Ureteral Obstruction on Renal Structure

Following ureteral obstruction, a number of factors result in morphologic changes to the kidney, including the increase in ureteral pressure, the

decrease in renal blood flow (ischemia), an invasion by macrophages and lymphocytes, and bacterial infection. The subsequent macroscopic structural changes that are found in the kidney depend on both the duration and degree of the obstruction.

Following acute complete obstruction initially, there is pelvicalyceal dilation, renal enlargement, and edema (Fig. 12-4, left panel). Microscopically, tubular dilation develops that predominantly affects the collecting duct and distal tubular segments (49), though cellular flattening and atrophy of proximal tubular cells can also occur. Glomerular structures are usually preserved initially, although Bowman's space may be dilated and may contain Tamm–Horsfall protein. Ultimately, some periglomerular fibrosis may develop.

In chronic partial obstruction a grossly hydronephrotic kidney develops with a widely dilated renal pelvis, with the renal papilla either flattened or hollowed out. The first structures to be affected are the ducts of Bellini. Subsequently, other papillary structures are damaged. Ultimately, there is an encroachment on renal cortical tissue, which in advanced cases may be reduced to a thin rim of renal tissue surrounding a large saccular ureteral pelvis (Fig. 12-4, right panel).

Prolonged obstruction results in the development of interstitial fibrosis with obliteration of nephrons. There is tubular proliferation and apoptosis, epithelial–mesenchymal transition (EMT), (myo)fibroblast accumulation, increased extracellular matrix deposition, and tubular atrophy. Ischemia as a result of the decreased renal blood flow contributes to the parenchymal damage after obstruction.

Both angiotensin II and transforming growth factor-β (TGF-β) appear to play an important pathogenetic role in the development of renal fibrosis following obstruction (50,51). Over the past two decades or so, experimental models of ureteral obstruction have been used to elucidate the mechanisms which underlie the development of fibrosis in CKD resulting in an in-depth understanding of the role played by signaling pathway networks and profibrotic cytokines in the regulation of kidney fibrosis (52).

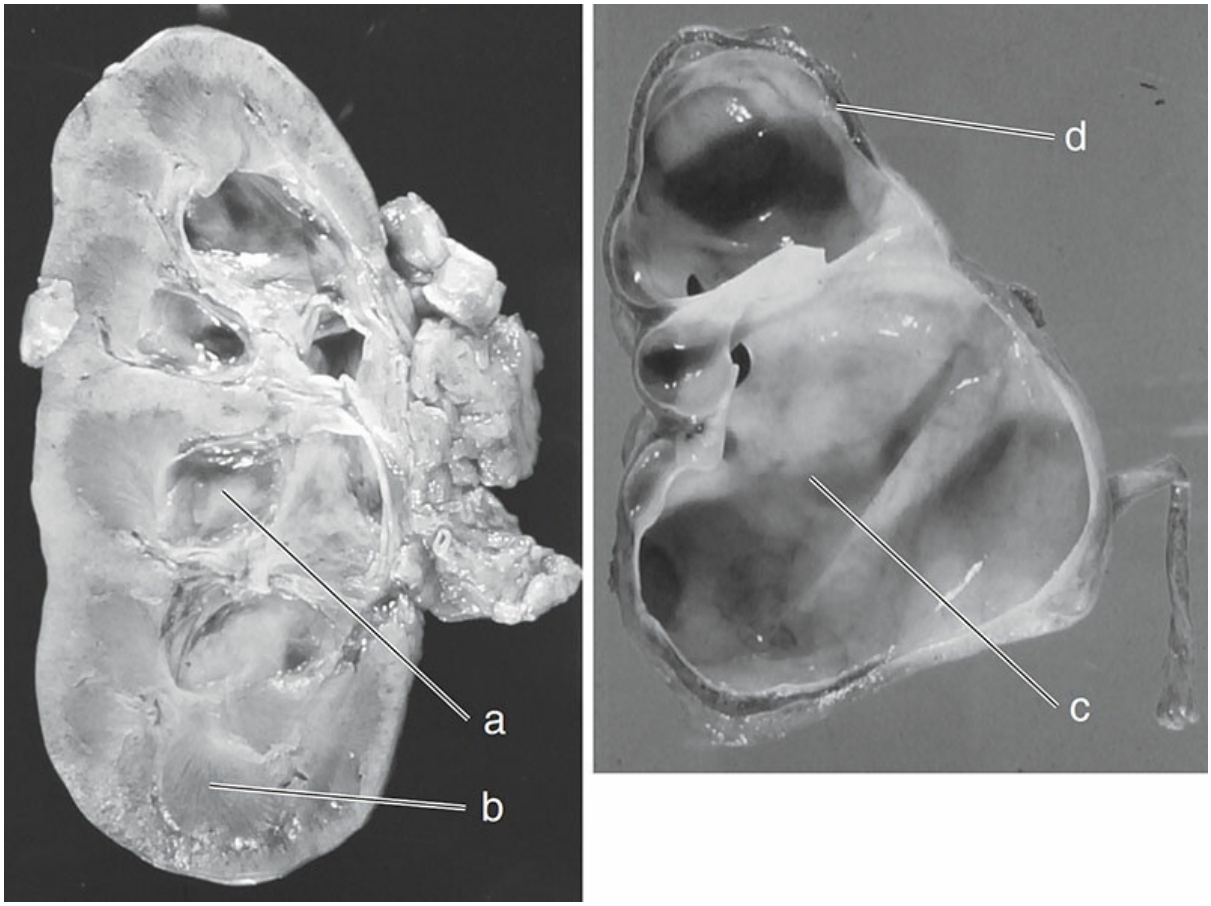

Figure 12–4 Kidney specimens demonstrating the contrasting macroscopic structural effects of complete short-term and partial long-term urinary tract obstruction. *Left Panel*: An acutely obstructed kidney showing a dilated pelvicalyceal system (*a*) and an edematous but well preserved renal parenchyma (*b*). *Right Panel*: A chronically partially obstructed kidney due to pelvic-ureteral junction obstruction showing gross dilatation of the pelvicalyceal system (*c*) and a thin and atrophic renal cortex (*d*).

Invading cells, particularly macrophages, by releasing inflammatory and growth factors, may contribute to interstitial cell proliferation and scarring and widening of the interstitium space. Superimposed bacterial infection (pyelonephritis) may play an additive role in the development of parenchymal fibrosis and in the pathologic changes that are observed (53).

The sequence of events whereby the acute functional and reversible alterations in kidney function following obstruction transform into chronic irreversible structural abnormalities involves a complex interplay between infiltrating and resident cells, the production of hormones, cytokines, and growth factors, as well as the modulation of matrix production and degradation. These factors are discussed below and are summarized in Figure 12-5.

THE DEVELOPMENT OF TUBULOINTERSTITIAL FIBROSIS

The tubulointerstitium occupies approximately 80% of total kidney volume. Renal interstitial fibrosis is a common consequence of long-standing obstructive uropathy (54) and develops because of an imbalance between extracellular matrix synthesis, matrix deposition, and matrix degradation. Typical findings include a widening of the interstitial space, a mononuclear cell infiltrate and a proliferation of interstitial cells in the renal parenchyma, and an increase in the renal synthesis of several extracellular matrix components (collagen types I, III, and IV; fibronectin; heparan sulfate proteoglycans) in the renal interstitium (55). These changes are associated with an increase in the level of messenger RNA (mRNA) for TGF-β_1 within the interstitium of the obstructed kidney after only short periods of obstruction suggesting that the events which lead to interstitial fibrosis are initiated promptly after the onset of obstruction (56).

Renal tubular cells in culture produce collagen types I, III, and IV, and the expression of collagen α_1 (type IV) mRNA increases in the tubules of the obstructed kidney. Therefore, renal tubule cells may contribute to the increased production of collagen IV in both tubular basement membrane and the interstitium, which in turn may contribute to alterations in tubular function in the obstructed kidney.

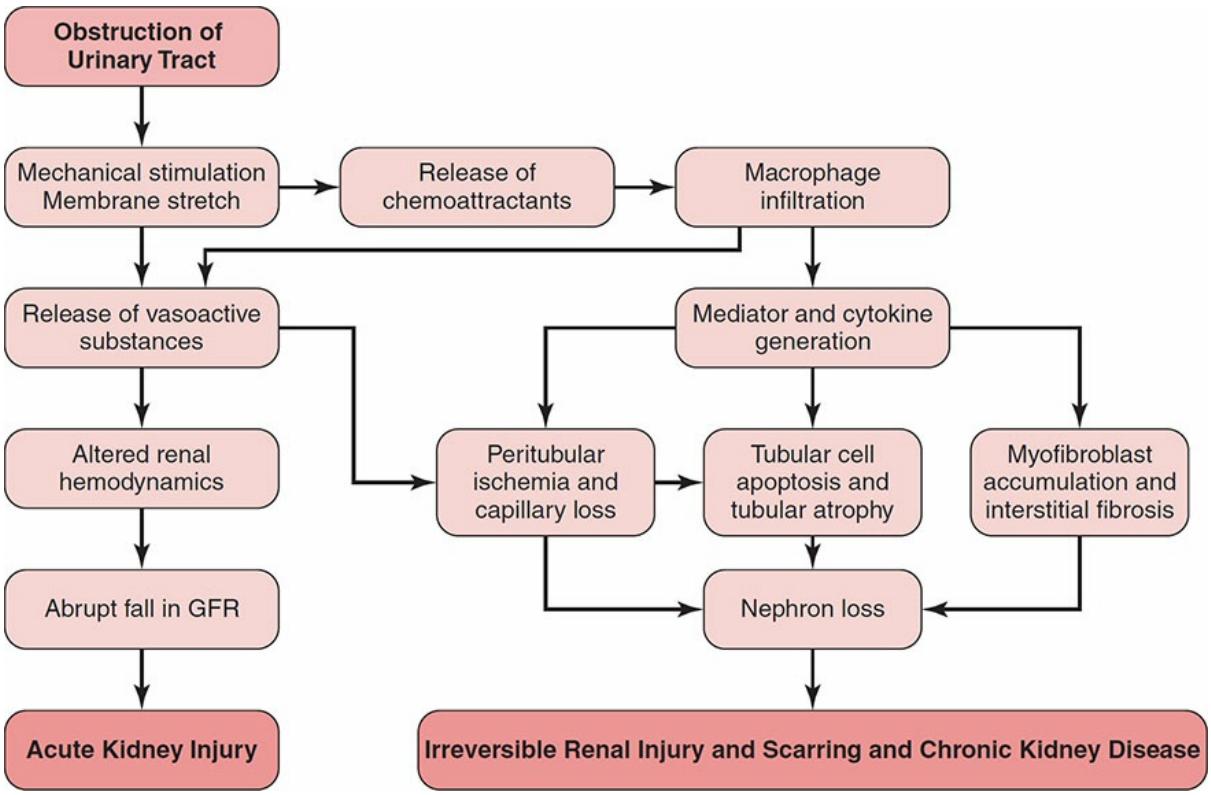

Figure 12–5 The sequence of events whereby the acute functional and reversible alterations in kidney function following obstruction transform into chronic irreversible structural abnormalities. Note the pivotal role of infiltrating macrophages in both modulating acute functional changes and promoting the development of irreversible structural damage and fibrosis.

At the same time, factors derived from infiltrating macrophages and T-lymphocytes stimulate fibroblast migration and proliferation in the interstitium of the obstructed kidney. Several cytokines secreted by infiltrating macrophages and T-lymphocytes act as chemoattractants and stimulate fibroblast proliferation. Interstitial fibroblasts produce collagens I, III, and IV, and therefore contribute to the increase in the production of collagens in the obstructed kidney. The substantial increase in collagens I and III in the interstitium of the obstructed kidney at 3 or 5 days after UUO is consistent with the increased cellularity caused by fibroblast proliferation and infiltrating mononuclear cells.

The increased expression of TGF-β_1 mRNA in the obstructed kidney is confined to tubular cells (56). TGF-β_1 has substantial effects on matrix protein production (52,57). It causes (a) an increase in the mRNA of extracellular matrix components, particularly the collagens; (b) a decrease in proteinases degrading these proteins; and (c) an increase in metalloproteinase inhibitors (Fig. 12-6).

In contrast, the amount of glomerular collagens I, III, and IV is unchanged as is mRNA for TGF-β_1 at day 5 after UUO (56), consistent with the finding that glomeruli appear normal by light microscopy after 7 days of obstructive nephropathy (58).

APOPTOSIS

Distinct patterns of cell proliferation and apoptosis have been described for tubular, interstitial, and glomerular cells, as well as infiltrating cells in chronic obstructive nephropathy. The development of interstitial inflammation and fibrosis following prolonged obstruction is accompanied by tissue loss and atrophy of the tubular epithelial cells (59,60). Apoptosis of renal tubular cells in chronic obstructive nephropathy increases rapidly, reaching 30-fold that of controls by 25 days of obstruction (61). This is accompanied by a decrease in the dry weight of the kidney, suggesting apoptosis participates in the tubular atrophy and renal loss observed in prolonged obstructive nephropathy.

Apoptosis is a prominent feature of obstruction to the urinary tract in

utero and leads to the loss of renal mass commonly observed in this condition. There is increasing evidence that apoptosis may also act as a trigger for the subsequent development of progressive interstitial fibrosis.

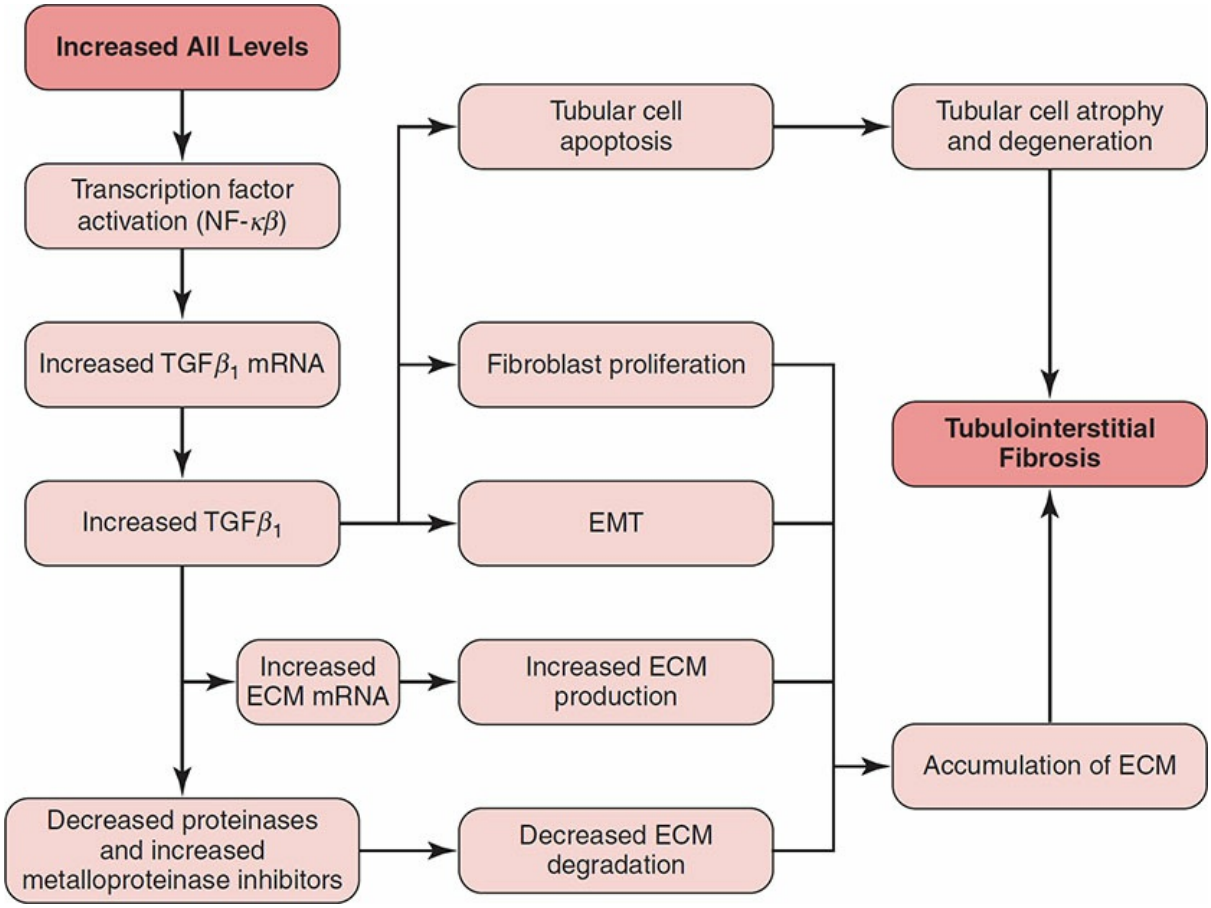

Figure 12–6 Pathogenesis of tubule-interstitial fibrosis in progressive renal disease. AII, angiotensin II; ECM, extracellular matrix; mRNA, messenger RNA; TGF-b_1, transforming growth factor beta-1; EMT, epithelial–mesenchymal transition.

INFILTRATING MACROPHAGES

Following ureteral obstruction, there is an increased synthesis and expression of adhesion proteins and chemoattractants in the kidney, which contribute to monocytic infiltration. Monocyte chemo-attractant protein-1 (MCP-1) mRNA and protein expression is increased within the proximal tubular epithelium of the obstructed but not the contralateral unobstructed kidney (62–64). The resulting macrophage infiltrate plays a pivotal role in the chronic tissue injury and fibrosis that result from prolonged ureteral obstruction (65) by releasing profibrogenic factors such as TGF-β and galectin-3 that promote progressive fibrosis (66). The critical role for infiltrating macrophages in the pathogenesis of the late structural changes

that occur after obstruction is demonstrated by the observation that macrophage depletion markedly limits the development of interstitial fibrosis. In addition to macrophages, the cellular infiltrate following obstruction also contains a number of T cells. The exact way in which the various cell types and local cytokine networks interact to modulate the fibrotic response is complex and may represent a final common pathway to the development of fibrosis in a number of renal diseases with different etiologies (67).

ANGIOTENSIN

Following ureteral obstruction, local angiotensin II generation can stimulate the production of TGF-β by tubular cells and promote the deposition of the type IV collagen and the development of tubulointerstitial fibrosis within the obstructed kidney (Fig. 12-6).

ACE inhibitors and angiotensin receptor antagonists exert a beneficial effect on the UUO model of experimental hydronephrosis (68,69). Administration of ACE inhibitors in rats with unilateral obstruction results in a decrease in interstitial volume, a marked decrease in the number of monocytes/macrophages infiltrating the renal parenchyma, a decrease in the expression of TGF-β, and lesser activation of nuclear factor kappa B (NF-κB) (69). In addition, there is a marked decrease in fibroblast proliferation and myofibroblast phenotype. The administration of ACE inhibitors after 5 days of established UUO prevents the progressive fibrosis that occurred in untreated rats from day 5 to day 10 of ureteral obstruction. Administration of an angiotensin I receptor antagonist has a similar effect, with the exception of the infiltration of the renal parenchyma by monocytes/macrophages and the expression of clusterin, which decreases in the kidney of rats with UUO treated with ACE inhibitors but not in rats treated with angiotensin I receptor antagonist (70). These differences may be explained by the effects of ACE inhibitors on nitric oxide, through the activation of bradykinin (71). Treatment with an angiotensin II receptor antagonist has no effect on interstitial volume, macrophage infiltration, expression of TGF-β, or fibroblast proliferation in rats with UUO (70). However, antagonism of the angiotensin II receptor decreases the appearance of a myofibroblast phenotype and markedly decreases the expression of clusterin, with an intermediate effect on the activation of NF-κB.

NF-κB

The activation of a number of genes associated with tissue inflammation and the development of fibrosis is controlled by the NF-κB family of transcription factors (67,72). NF-κB has been shown to be activated during experimental ureteral obstruction (73). Enalapril, given to rats with ureteral ligation, significantly decreases the ability of proteins extracted from the nucleus to bind to an NF-κB consensus oligonucleotide compared with similar extracts obtained from kidneys of untreated animals (74). This suggests ACE inhibitors might protect against the development of renal fibrosis by directly decreasing activation of NF-κB, in addition to their well-known hemodynamic effects.

REACTIVE OXYGEN SPECIES

A growing body of evidence supports the concept that oxidative stress, resulting in generation of reactive oxygen species (ROS), plays a critical role in the development and progression of renal fibrosis by inducing extracellular matrix accumulation (75). ROS can mediate the profibrotic effects of TGF-β_1 and also activate intercellular adhesion molecule 1 (ICAM-1) and thus may play a central role in the mediation of inflammatory cell proliferation and extracellular matrix accumulation. In obstructive nephropathy, oxidants generated by infiltrating leukocytes and intrinsic renal cells may account for some of the functional and morphologic changes observed. Probucol, an antioxidant and lipid-lowering agent, improves GFR and renal plasma flow both at 4 hours and 3 days after release of 24 hours of bilateral obstruction (76), whereas lipid lowering with lovastatin, which is devoid of antioxidant properties, has no effect.

A decrease in mRNA and protein expression of cellular antioxidant enzymes and increased generation of ROS may play an integral role in the development of tubulointerstitial injury and fibrosis associated with experimental hydronephrosis. As early as 24 hours after UUO, levels of total cortical mRNA for catalase and Cu-ZnSOD are significantly decreased in the obstructed kidney and there is a decreased immunohistochemical staining intensity for Cu-ZnSOD and catalase protein in the cortical tubules (77). Thus, in addition to an increased generation of ROS within the obstructed kidney cortex, there is impairment of normal antioxidant defense mechanisms.

The importance of oxidative stress in the development of renal fibrosis has been recently confirmed by studies on intermedin, a peptide inhibitor of oxidative stress. Overexpression of intermedin within the kidney was

able to attenuate the increase in oxidative stress, macrophage infiltration, tubular injury, and fibrotic response to ureteral obstruction (78).

HOMEOSTATIC FACTORS

The majority of studies have investigated the role that the upregulation of hormones and cytokines plays in the development of tubulointerstitial fibrosis. However, a decrease in the production of growth and homeostatic factors, which are normally endogenously produced by the kidney to downregulate the fibrotic process, may also be important in the development of fibrosis.

The expression of preproepidermal growth factor is suppressed in the kidney with an obstructed ureter in both the neonatal and adult rat (79,80). Treatment with epidermal growth factor significantly reduces tubule cell apoptosis, blunts tubule atrophy, and preserves renal function when the obstruction is relieved.

Endogenous insulin-like growth factor-1 (IGF-1) expression is not changed during UUO in neonatal rats. Although IGF-1 treatment does not affect the suppression of nephrogenesis or tubule cell proliferation seen in obstructed neonatal rat kidneys, it does significantly blunt tubule cell apoptosis, tubule atrophy, and interstitial collagen deposition following relief of obstruction (81), suggesting that IGF-1 treatment could offer another means of preserving the capacity of renal function once flow is reestablished.

In a mouse model of unilateral obstruction, treatment with recombinant human hepatocyte growth factor (HGF) attenuates apoptosis and TGF-β expression, whereas treatment with an HGF-neutralizing antibody increases TGF-β expression, decreases tubule cell proliferation, and accelerates apoptosis, suggesting that a reduction in endogenous HGF could account for progression of renal fibrosis in tubulointerstitial disease (82).

Bone morphogenetic protein-7 (BMP-7) treatment significantly decreases renal injury in a rat model of UUO both when treatment is initiated at the time of injury (83) and when administered after renal fibrosis has begun (84).

Tubular epithelial cells are one of the major sites of active vitamin D synthesis. Paricalcitol, a synthetic vitamin D analogue, has been shown to significantly attenuate the development of renal interstitial fibrosis in mouse kidney after ureteral obstruction. It reduces interstitial volume, decreases collagen deposition, and lowers mRNA expression of

fibronectin and type I and type III collagens. In addition, paricalcitol suppresses the expression of renal TGF-β_1 and its type I receptor and inhibits cell proliferation and apoptosis after obstructive injury. In vitro, paricalcitol was able to block EMT. These data suggest that paricalcitol is able to ameliorate renal interstitial fibrosis in obstructive nephropathy, possibly by preserving tubular epithelial integrity through suppression of EMT (85).

Heat shock protein (HSP72) has also been shown to ameliorate renal tubulointerstitial fibrosis in obstructive nephropathy by inhibiting both renal tubular epithelial cell apoptosis and EMT (86).

An understanding of the complex interactions between profibrotic cytokines and homeostatic factors in modulating the development of tubulointerstitial inflammation and fibrosis, EMT, and tubular cell apoptosis following obstruction has contributed to the development of putative therapeutic targets for blunting the development of progressive fibrotic renal disease (87).

Clinical Findings in Urinary Tract Obstruction

Obstruction of the urinary tract is a common and potentially reversible cause of AKI, and therefore it is important to diagnose and treat it promptly to minimize the chances of long-term chronic damage to the kidney.

Obstruction of the urinary tract can present with a wide range of clinical findings, depending on the site, degree, and duration of obstruction. The clinical manifestations of upper and lower urinary tract obstruction differ. Mechanical obstruction of the urinary tract, causing pain, and lower urinary tract symptoms (prostatism) are common presenting complaints. Symptoms can also result from the complex alterations in glomerular and tubular function that may occur in obstructive nephropathy. However, it is important to note that obstructive uropathy and hence obstructive nephropathy can occur without symptoms. In some cases, the symptoms may be related to urinary tract infection or the underlying pathologic process responsible for the development of obstructive uropathy such as tumors or metastases. Obstruction of the urinary tract must be considered in the differential diagnosis of any patient with renal impairment.

SYMPTOMS

Pain

Pain is a common presenting complaint in patients with obstructive uropathy, particularly in those with ureteral calculi or where the obstruction has developed rapidly. The pain is believed to result from stretching of the collecting system or the renal capsule, with its severity correlating with the degree of distention and not with the degree of dilation of the urinary tract. Occasionally, the location of the pain helps to determine the site of obstruction. With upper ureteral or pelvic obstruction, flank pain and tenderness typically occur, whereas lower ureteral obstruction causes pain that radiates to the groin, the ipsilateral testicle, or the labia. Acute high-grade ureteral obstruction may be accompanied by a steady and severe crescendo flank pain radiating to the labia, the testicles, or the groin (“classic” renal colic). The acute attack may last less than half an hour, or as long as a day. In contrast, pain radiating into the flank during micturition is said to be pathognomonic of VUR. By comparison, patients with a chronic, slowly progressive obstruction may have no pain or minimal pain during the course of their disease. In such patients, any pain that does occur is rarely colicky in nature. In PUJ obstruction, pain may only occur after fluid loading or the use of diuretics to promote a high urine flow rate.

Hematuria

Calculi may cause trauma to the urinary tract uroepithelium and result in either macroscopic (visible) or microscopic (nonvisible) hematuria. Any neoplastic lesion that obstructs the urinary tract, especially uroepithelial malignancies, may bleed, resulting in macroscopic hematuria. Urinary tract bleeding may also result in obstruction, giving rise to clot colic when in the ureter or clot retention when in the bladder.

Alterations in Urine Output

Patients with complete bilateral obstruction or obstruction in a single functioning kidney present with anuria and AKI. In contrast, partial obstruction may present with polyuria and polydipsia (88) as a result of acquired resistance to ADH. Alternatively, there may be a fluctuating urine output, alternating from oliguria to polyuria. A pattern of alternating oliguria and polyuria or the presence of anuria strongly suggests

obstructive uropathy.

Lower Urinary Tract Symptoms

Obstructive lesions of the bladder neck or bladder pathology may cause a decrease in the force or caliber of the urine stream, intermittency, postmicturition dribbling, hesitancy, or nocturia. Urgency, frequency, and urinary incontinence can result from incomplete bladder emptying. Such symptoms commonly result from prostatic hypertrophy and are frequently referred to as prostatism, but they are not pathognomonic of this condition.

Urinary Tract Infection

Urinary tract infections are common and in most cases will not be associated with obstruction to the urinary tract (89). However, a urinary tract infection in neonates, young children of either sex or men, recurrent or persistent infections in women, or infections with unusual organisms, such as Pseudomonas species should prompt further investigation to exclude obstruction. Obstruction should also be excluded following a single episode of upper urinary tract obstruction (acute pyelonephritis). The presence of ongoing obstruction can make the effective eradication of the infection difficult. In a study of adult males with simple or recurrent urinary tract infections, a significant underlying lower urinary tract abnormality, mainly bladder outflow obstruction, was found in 80% of cases (90).

Infection tends to be more common with obstruction of the lower urinary tract (below the ureterovesical junction) and presents with symptoms of cystitis such as dysuria and frequency. The increase of residual urine in the bladder (urine is an excellent culture medium) and altered properties of the bladder that facilitate bacterial adhesion and growth predispose to infection. Alterations in the glycoprotein composition of epithelial cells of the bladder may explain the greater predisposition to infection in certain patients with urinary tract obstruction than in others.

Although obstruction of the upper urinary tract is not necessarily accompanied by infection, when it occurs (acute pyelonephritis) life-threatening systemic sepsis may result.

Infections of the urinary tract with a urease-producing organism such as *Proteus mirabilis* predispose to stone formation. These organisms generate ammonia, which results in urine alkalinization and favors the

development of magnesium ammonium phosphate (struvite) stones. Struvite calculi can fill the entire renal pelvis to form a staghorn calculus that eventually leads to loss of the kidney if untreated. Thus, stone formation and papillary necrosis can also be a consequence of urinary tract obstruction as well as a cause of obstruction.

Obstruction in Neonates or Infants

Oligohydramnios at the time of delivery should raise the suspicion of obstructive uropathy, as should the presence of congenital anomalies of the external genitalia. Nonurologic anomalies such as ear deformities, a single umbilical artery, an imperforate anus, or a rectourethral or rectovaginal fistula should prompt investigation for urinary tract obstruction. The urinary tract also should be examined in infants born with an imperforate anus or a rectourethral or rectovaginal fistula. The existence of a neurogenic bladder with associated obstructive uropathy should be suspected in infants with neurologic abnormalities.

However, the symptoms of obstructive uropathy in neonates and infants are frequently nonspecific and may not be suspected until failure to thrive, voiding difficulties, fever, hematuria, or symptoms of renal failure appear.

The advent of routine antenatal scanning has improved the early diagnosis of congenital anomalies of the kidney and urinary tract, and antenatal hydronephrosis is now one of the most commonly detected birth defects. If congenital urinary tract obstruction goes undiagnosed, the child may present in the postnatal period with failure to thrive, voiding difficulties, fever, hematuria, or symptoms of renal failure. Complete obstruction to the renal tract has rapid and devastating consequences on renal development but is relatively rare. Partial obstruction, for example from ureteropelvic junction obstruction, is more frequently observed but can still cause longer term complications such as hypertension and CKD (91). The development of accurate antenatal diagnosis offers the prospect of offering prompt intervention to relive obstruction to those infants at risk of developing CKD (92). However, the benefit of such strategies still requires careful evaluation to identify the impact on important long-term outcomes such as proteinuria, hypertension, and CKD (93).

CLINICAL EXAMINATION

General Examination

Physical examination can be completely normal. Some patients with upper urinary tract obstruction may have flank tenderness. Kidney size may increase significantly, particularly in long-standing obstruction. Patients may note increased abdominal girth, and a palpable flank mass may be found. Muscle rigidity over the kidney may be found, and rebound tenderness may be elicited, particularly if acute infection is present. Marked hydronephrosis may present as a flank mass on physical examination, particularly in children with hydronephrosis who are younger than 2 years.

Lower urinary tract obstruction causes a distended, palpable, and occasionally painful bladder. A rectal examination and, in women, a pelvic examination should be performed because they may reveal a local malignancy or prostatic enlargement.

Evidence of an underlying pathologic process responsible for the development of obstructive uropathy, such as tumors or metastases from distal tumors, may be detected.

Blood Pressure

Hypertension may occur in patients with either unilateral or bilateral acute or chronic hydronephrosis. This may be the result of either an increase in ECF volume, owing to decreased sodium excretion, or to an abnormal release of renin and increased generation of angiotensin II. In patients with bladder outflow obstruction and bilateral hydronephrosis, the hypertension typically resolves promptly with the diuresis that occurs after the insertion a urinary catheter or corrective surgery, suggesting that the hypertension was volume dependent. In addition, the concentrations of renin in renal venous blood and peripheral venous blood are normal in hypertensive patients with bilaterally hydronephrotic kidneys.

In contrast, elevated values for renal vein renin have been found in unilaterally hydronephrotic kidneys and after appropriate surgery, the hypertension abates, and the renin values return to normal (94).

Animal studies have demonstrated an increase in renin release following acute ureteral obstruction (95). However, with prolonged unilateral ureteral occlusion in animals, the renin release is not sustained and the peripheral renin is normal. Thus the mechanism of established hypertension in the setting of long-standing unilateral obstruction is more complex.

Occasionally, in patients with partial urinary tract obstruction, hypotension occurs as a result of polyuria and volume depletion.

LABORATORY FINDINGS IN URINARY TRACT OBSTRUCTION

Urine Abnormalities

Urinalysis may show hematuria, bacteriuria, pyuria, crystalluria, and low-grade proteinuria, depending on the cause of obstruction. However, urinalysis is commonly completely negative in obstructive nephropathy. In the acute phase of obstruction, urinary electrolytes are similar to those seen in a "prerenal" state, with a low urinary sodium (<20 mmol/L), a low fractional excretion of sodium (<1%), and a high urinary osmolality (>500 mOsm/kg). However, with more prolonged obstruction, there is a decreased ability to concentrate the urine and an inability to reabsorb sodium and other solutes. These changes are particularly marked after the release of chronic obstruction and give rise to the syndrome commonly referred to as postobstructive diuresis.

Serum Electrolyte Abnormalities

Hyperkalemic hyperchloremic acidosis (renal tubular acidosis type 4) may be a clinical manifestation of partial obstruction of the urinary tract (96) (see pathophysiology section of this chapter for the mechanisms).

Children with partial obstructive uropathy may develop hypernatremia because polyuria causes a greater loss of water than sodium.

Renal Impairment

Bilateral obstruction of the urinary tract, or obstruction to a single functioning kidney, will result in AKI with a rapidly rising urea and creatinine. Obstruction should always be considered when AKI presents with complete anuria or if periods of anuria alternate with periods of polyuria.

If left untreated urinary tract obstruction will result in CKD, and urinary tract obstruction should always be considered in patients with CKD and no previous history of renal disease and a relatively benign urinary sediment. Elderly men can present with advanced CKD and hydronephrosis secondary to bladder outflow obstruction despite remarkably few lower urinary tract symptoms, and in patients with retroperitoneal fibrosis in whom the onset of obstruction is slow and progressive, far-advanced CKD may also be the initial presenting finding.

Urinary tract obstruction may also accelerate progression of an underlying parenchymal renal disease of another etiology so obstruction should also be excluded in patients with known renal disease who develop an abrupt decrease in renal function that is otherwise unexplained.

Polycythemia

Polycythemia has been reported in a few instances of hydronephrosis and is probably related to increased production of erythropoietin by the obstructed kidney. In experimental animals, unilateral hydronephrosis results in elevated plasma levels of erythropoietin that precede the increase in hemoglobin levels.

DIAGNOSIS OF URINARY TRACT OBSTRUCTION

Prompt diagnosis of urinary tract obstruction is essential to allow treatment to limit any long-term adverse consequences. Symptoms such as “renal colic” may suggest the diagnosis and prompt appropriate investigation. However, there should be a high index of suspicion of urinary tract obstruction in any patient with unexplained AKI or CKD. The diagnostic approach has to be tailored to the clinical presentation (Fig. 12-7), but a careful history and thorough physical examination are mandatory in all patients.

A history of similar symptoms, the presence or absence of lower urinary tract symptoms or urinary tract infection, and the kinds of drugs ingested should be noted. Review of hospital records may reveal abrupt changes in urine output with anuria being suggestive of complete obstruction. However, polyuria may occur in partial obstruction and may mimic that of patients with nephrogenic diabetes insipidus. In children, the manifestations of obstructive uropathy may include gastrointestinal symptoms such as nausea, vomiting, and abdominal pain.

Physical examination with particular reference to the flank and abdomen is important.

Laboratory analysis of urine and serum as outlined above are mandatory. However, a definitive diagnosis of obstruction requires imaging of the renal tract to confirm the diagnosis, elucidate the cause, and plan treatment.

IMAGING TECHNIQUES FOR THE DIAGNOSIS OF

URINARY TRACT OBSTRUCTION

As the sites, causes, and consequences of obstruction to the renal tract are so variable, no single imaging investigation is able to diagnose renal tract obstruction with certainty. Modern imaging technology particularly computed tomography (CT) scanning and magnetic resonance (MR) urography, have improved the ability to accurately diagnose both the site and the cause of obstruction. The protocols which are used in the investigation of obstruction will depend on the local expertise, the availability of resources, and concerns about radiation exposure (97), and it is important to remember that older imaging techniques can still be used effectively to evaluate patients with obstructive uropathy.

Generally, the approach to the patient with suspected obstruction may require the complementary use of a number of different imaging techniques, and no single imaging investigation should be relied on to definitively exclude obstruction, especially if the clinical suspicion of obstruction is high.

Several radiologic techniques can be used to infer the presence of upper urinary tract obstruction from the finding of dilatation of the pelvicalyceal system (hydronephrosis). However, it must be remembered that not all dilated collecting systems represent obstruction.

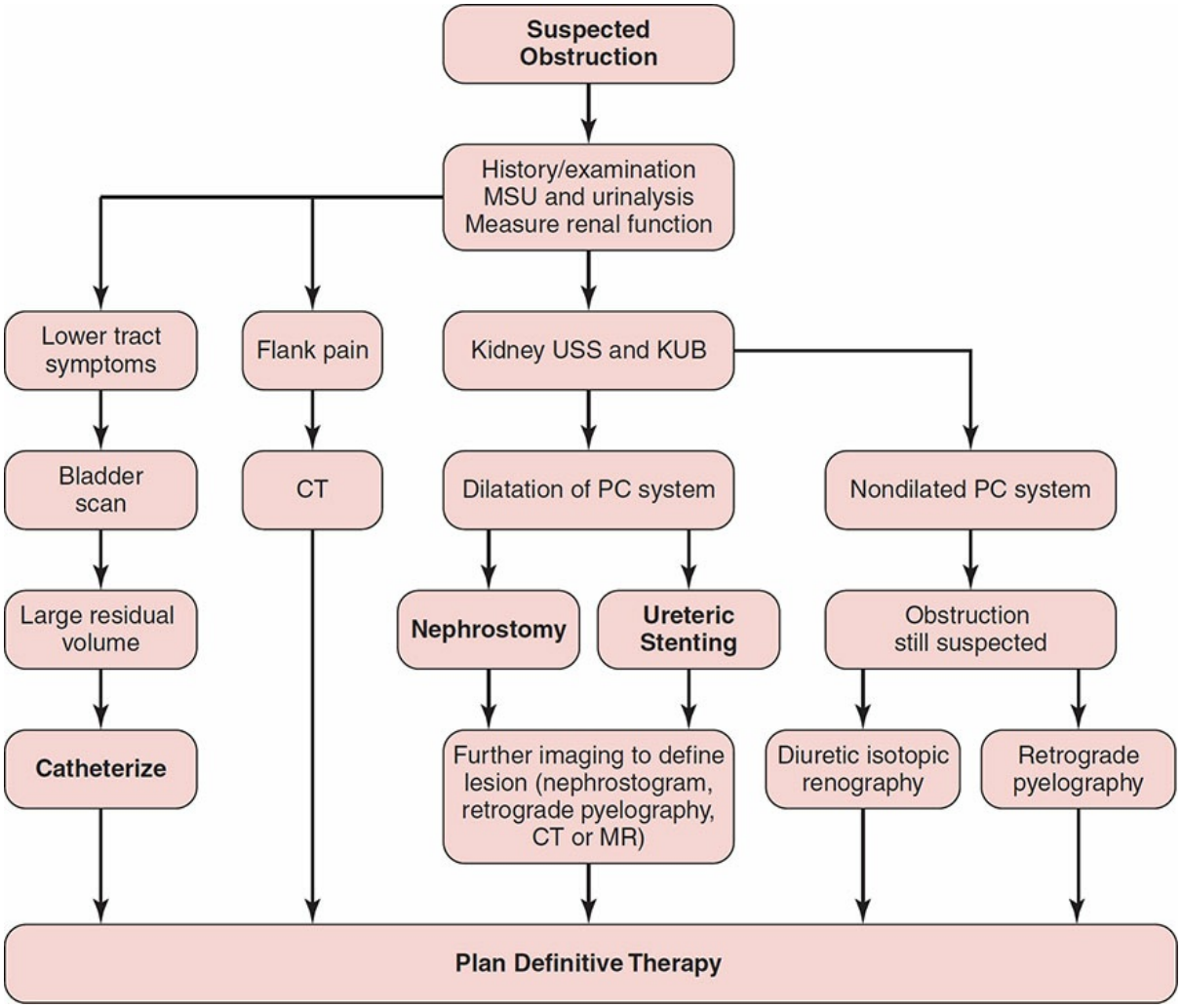

Figure 12–7 Algorithm demonstrating an approach to the investigation and management of suspected urinary tract obstruction. The initial investigations (*boxes*) are dictated by the history and examination. The patient pathway allows rapid relief of the obstruction by nephrostomy or ureteric stenting, while a definitive diagnosis and treatment plan are made. CT, computed tomography; MR, magnetic resonance; USS, ultrasound scan.

Plain Abdominal X-Ray

A plain abdominal X-ray (or kidneys, ureters, and bladder [KUB]) provides information on renal and bladder morphology, such as size differences between the two kidneys or a large bladder, suggestive of outlet obstruction. It can frequently demonstrate renal calculi, since about 90% of calculi are radiopaque.

Intravenous Urography

Historically, intravenous urography (IVU) was the first-line investigation

for suspected upper urinary tract obstruction but is now rarely used as a first-line investigation having been superseded by the use of ultrasound, CT, and MR.

In patients with normal renal function, IVU can usually define both the site and the cause of the obstruction. However, the excretion of contrast may be poor or delayed in patients with low GFR because of a decreased filtered load of contrast, and films as long as 1 day after radiocontrast injection may be required. In addition, the contrast media is potentially nephrotoxic to an already damaged kidney, particularly in patients older than 60 years and those with diabetes mellitus, preexisting CKD, or dehydration.

Oblique films of the bladder and urethra during voiding (excretory cystogram) may be used to evaluate the site of any lower urinary tract obstruction.

Ultrasonography

Ultrasonography is a noninvasive test used as a screening procedure for obstruction. Ultrasonography can define renal size and demonstrate calyceal dilation (98) (Fig. 12-8), but its sensitivity and specificity depend heavily on the expertise of the operator. Ultrasound is rarely able to detect the cause of obstruction, since pathology within the ureter is difficult to demonstrate and tiny stones will not generate acoustic shadows. However, unilateral hydronephrosis suggests obstruction of the upper urinary tract by stones, blood clots, or tumors. Bilateral hydronephrosis is more likely to result from a pelvic problem obstructing both ureters or obstruction of the bladder outlet, in which case the bladder will also be enlarged. Ultrasonography is often combined with a KUB to ensure that ureteral stones or small renal stones are not overlooked.

Ultrasonography produces false-negative results in cases of nondilated obstructive uropathy. Immediately after acute obstruction (<24 hours), the relatively noncompliant collecting system may not have dilated such that an ultrasound examination may be normal. Furthermore, if urine flow is low, as in severe dehydration or renal failure, there may be little dilation of the urinary tract. Dilatation may also be absent in slowly progressive obstruction when the ureters are encased by fibrous tissue (as in retroperitoneal fibrosis) or by tumor. The acoustic shadow of a staghorn calculus can also mask dilation of the upper urinary tract. The sensitivity of ultrasound for diagnosing obstruction can be improved by measuring the resistive index using color Doppler sonography. A resistive index >0.7

reflects the increased vascular resistance present in obstruction and effectively discriminates between obstructed and nonobstructed kidneys (98). Ultrasound techniques are particularly useful when it is important to avoid the use of ionizing radiation such as with pregnant women and children and for the follow-up of patients requiring repeated imaging, such as after extracorporeal shock wave lithotripsy (ESWL).

Even in experienced hands, ultrasound may have a significant false-positive rate, especially if minimal criteria are adopted to diagnose obstruction. The echogenicity produced by multiple renal cysts may be mistaken for hydronephrosis on ultrasonography, and anatomic variations of the pelvicalyceal system (e.g., extrarenal pelvis) may be interpreted as dilatation of the urinary tract. There are also a number of nonobstructive causes of upper renal tract dilation, for example, VUR.

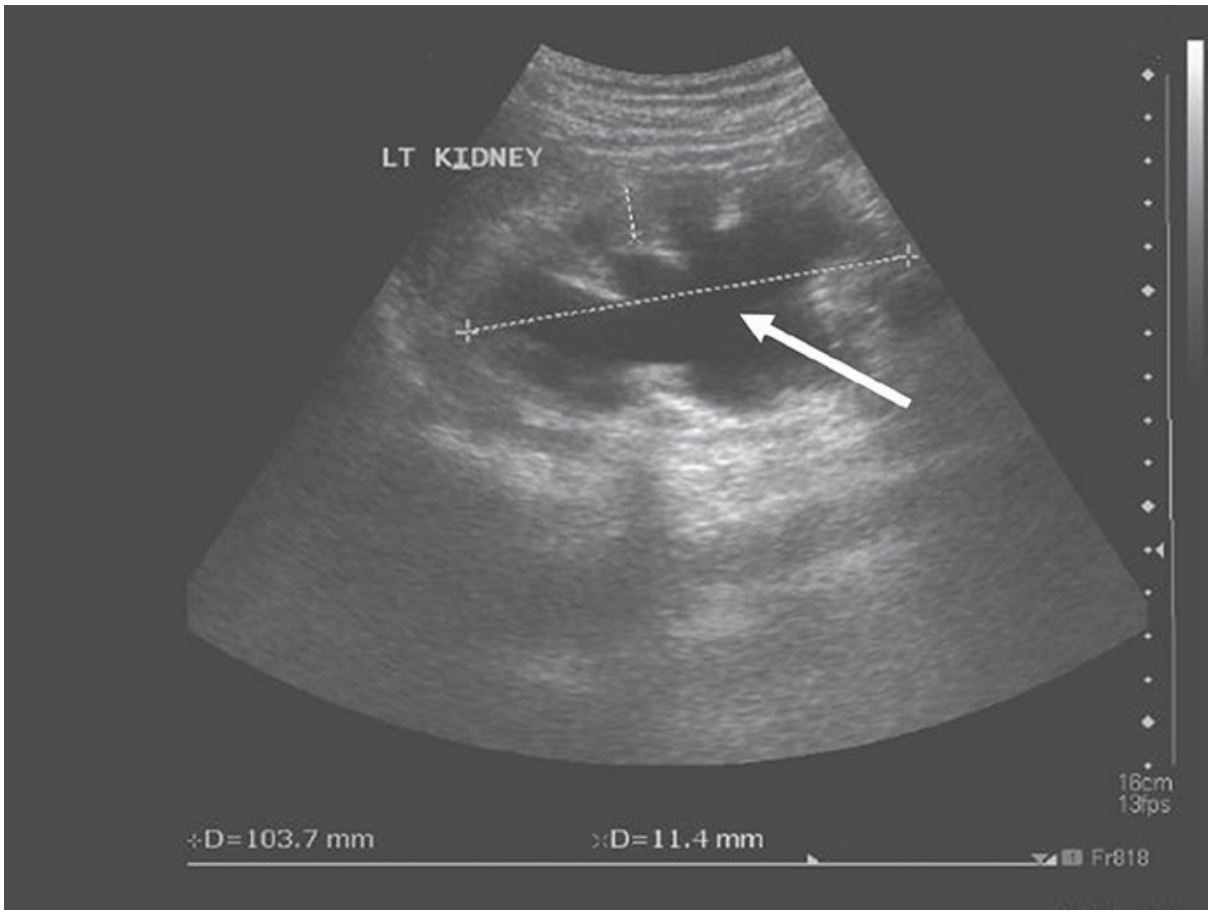

Figure 12–8 Renal ultrasound scan showing a hydronephrotic kidney. There are markers to define renal length and cortical width. There is marked dilation of the pelvicalyceal system with clubbing of the calyces (*arrow*). This is suggestive (but in isolation not diagnostic) of obstruction to the urinary tract.

Ultrasound may also provide useful information about the lower urinary tract. Pre and postmicturition volumes can be measured to assess

bladder emptying and the bladder wall can be assessed for wall thickening and trabeculation which suggests the presence of long-term bladder outflow obstruction. Using a full bladder as an acoustic window, ultrasound may also be used to assess the prostate in males and gynecologic structures in females.

In adults older than 60 years, 50 to 100 mL of residual urine may remain after each voiding because of the decreased contractility of the detrusor muscle. In chronic retention, ultrasound of the bladder may show massive increase in bladder capacity with very large (sometimes >500 mL) postmicturition volumes. This suggests significant bladder outflow obstruction predisposing to recurring urinary tract infections and requires further urologic investigation and treatment.

Ultrasound is used for follow-up in neonates with hydronephrosis diagnosed antenatally. If there is no calyceal dilatation (grade 1–2 hydronephrosis) surveillance may be continued, but the presence of increasingly severe pelvicalyceal dilatation (grade 3–5 hydronephrosis) requires further investigation with voiding cystourethrography to distinguish between megaureter resulting from obstruction or reflux and diagnose posterior urethral valves and ureteropelvic junction obstruction.

Computed Tomography

Noncontrast-enhanced spiral CT scanning is often the primary imaging modality for the evaluation of patients who present with undifferentiated acute flank pain (99,100) and can very accurately detect renal stones because of their high density (Fig. 12-9). However, there is concern that this modality is being overused both from the point of view of the resultant exposure to ionizing radiation (CT represents only 11% of radiologic examinations but is responsible for two-thirds of the ionizing radiation associated with medical imaging in the United States with recent estimates suggesting that there will be 12.5 cancer deaths for every 10,000 CT scans) and the cost involved.

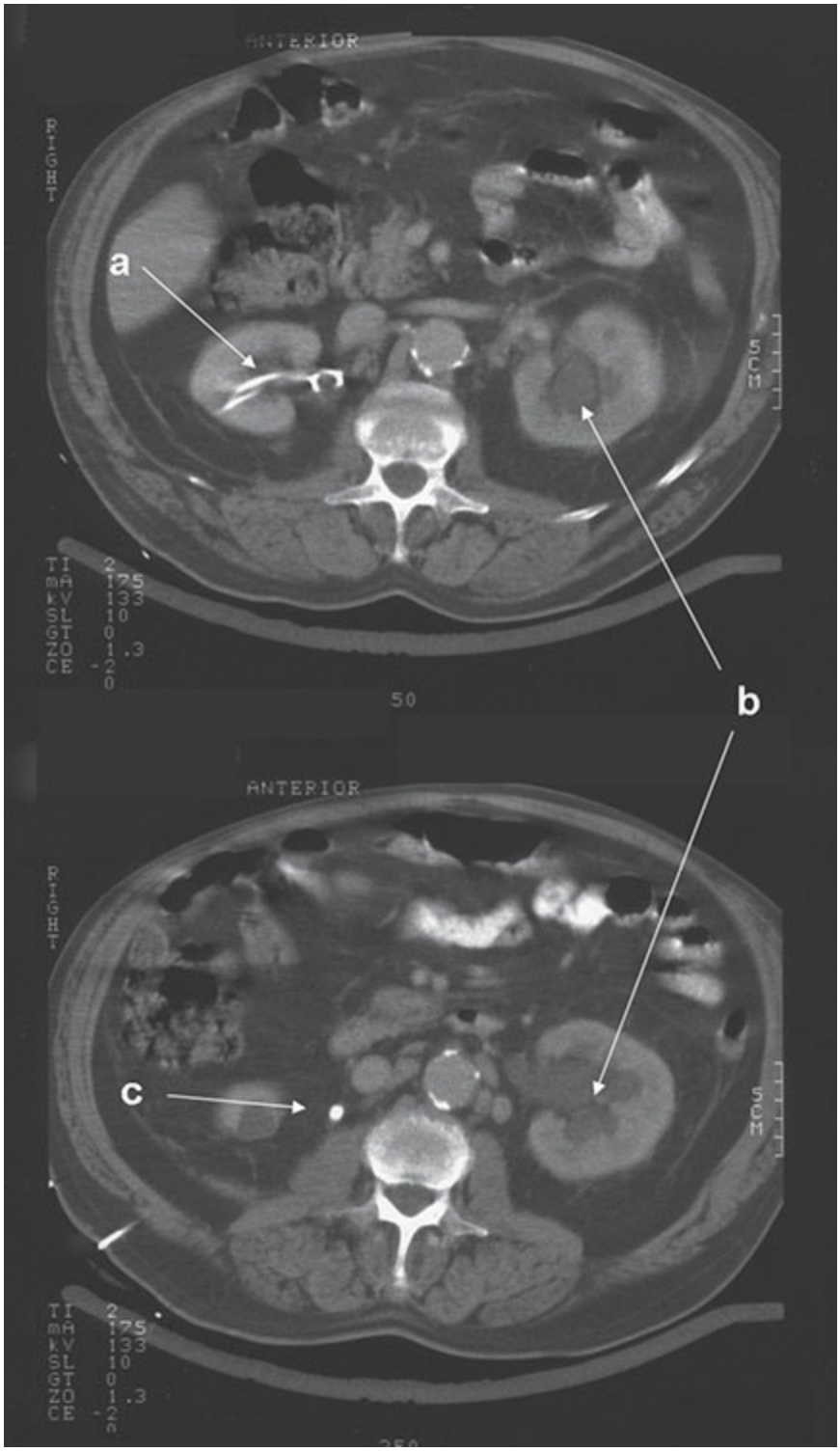

Figure 12–9 Abdominal CT scan of a patient with BUO secondary to renal calculi. In the upper panel, a nephrostomy has been inserted into the right kidney (*a*) to decompress the obstruction, while the left kidney remains obstructed and hydronephrotic (*b*). The lower panel demonstrates a ureteric calculus (*c*). BUO, bilateral ureteral obstruction; CT, computed tomography.

In patients where obstruction has been detected by other modalities, CT can be particularly useful in determining the site and nature of the

obstructing lesion, especially when it is extrinsic to the urinary tract. CT demonstrates retroperitoneal pathology such as para-aortic and paracaval lymphadenopathy, while retroperitoneal fibrosis is evident as increased attenuation within the retroperitoneal fat, with encasement of one or both ureters. Hematomas, primary ureteral tumors, and polyps are also detectable. Enhancements to the technique such as virtual CT pneumoendoscopy have been described, which may provide an important adjunctive diagnostic aid for urologic pathologies, thus avoiding the need for urinary tract endoscopy (101). The diagnostic potential of CT is also enhanced by the use of contrast, but concerns over nephrotoxicity limit its use in patients with renal impairment. The main drawback of CT remains the considerable exposure to ionizing radiation, making it unsuitable when frequent repetitive examinations may be required.

Magnetic Resonance Urography

MR urography (combined with KUB) can diagnose ureteral obstruction due to renal calculi with similar accuracy to spiral CT scanning but without exposure to a contrast medium or ionizing radiation and is increasingly being used to evaluate obstruction to the renal tract. The technique has less observer variability and is more accurate than CT in detecting indirect evidence of obstruction such as perirenal fluid (102). MR urography can rapidly and accurately depict the morphologic features of dilated urinary tracts and provide information regarding the degree and level of obstruction (103). MR urography also allows functional as well as anatomic parameters of the obstructed kidneys to be determined, as there is an excellent correlation between the GFR determined by MR urography and the isotope GFR (104). However, there is a possible risk of nephrogenic systemic fibrosis from gadolinium exposure in patients with a GFR <30 mL/minute which restricts its use in patients with significant renal impairment.

MR urography is a particularly attractive imaging modality for the evaluation of hydronephrosis in children as it provides both anatomic and functional data and can indicate whether the hydronephrosis is compensated (symmetrical changes in signal intensity of the nephrogram) or decompensated (105). Signs of decompensation (acute on chronic obstruction) include edema of the renal parenchyma, a delayed and increasingly dense nephrogram, a delayed calyceal transit time, and a >4% difference in the calculated differential renal function.

Retrograde Pyelography

Retrograde pyelography involves the retrograde injection of radiocontrast material and is used to visualize the ureter and the collecting system. This technique may be helpful when nondilated urinary tract obstruction is suspected or when there is a history of allergic reactions to contrast material. Urinary tract infection which may become overwhelming during instrumentation of the renal tract is a contraindication to retrograde pyelography.

Retrograde pyelography can identify both the site and the cause of the obstruction (106). It is helpful to include a postdrainage film, which is generally obtained 10 minutes after the retrograde injection of the radiocontrast. If the contrast medium does not persist in the collecting system, obstruction is unlikely, although residual contrast material can sometimes remain on a postdrainage film in a patient with a dilated but nonobstructed ureter if they are dehydrated and supine.

Instrumentation can introduce infection into the urinary tract; so should an obstructing lesion be found, it is essential to provide prompt adequate drainage to reduce any risk of overwhelming infection. It may be possible to effectively relieve the obstruction by placing a stent endoscopically in the ureter during the same procedure.

Isotopic Renography (Renal Scintigraphy)

Isotopic renography can be used to determine the functional significance of dilation of the collecting system (107,108). It requires the intravenous injection of the radionuclide technetium-99m mercaptoacetyltriglycine (99mTc-MAG3), combined with intravenous furosemide, administered 20 to 30 minutes after injection of the isotope (diuretic isotopic renography). Normally, there is a rapid washout of the isotope from the kidney. If there is functional dilatation of the collecting system, the isotope will be retained in the kidney. However, if there is no functional obstruction, the administration of the diuretic should cause a rapid washout of the isotope. Persistence of the isotope suggests that the system is not only dilated but also obstructed. Idealized tracings are summarized in Figure 12-10. Tracing I is a patient with a normal urinary tract. Tracing II strongly suggests obstruction because the radioisotope is retained in the pelvis and collecting system and there is no excretion following furosemide administration. Tracing III suggests dilatation without obstruction, because after furosemide administration there is rapid disappearance of the isotope.

The isotopic renogram is relatively noninvasive and can be performed in most hospitals and clinics but is seldom the definitive test. Markedly reduced renal function limits the usefulness of this test because the diuretic response to furosemide may be absent, making interpretation difficult.

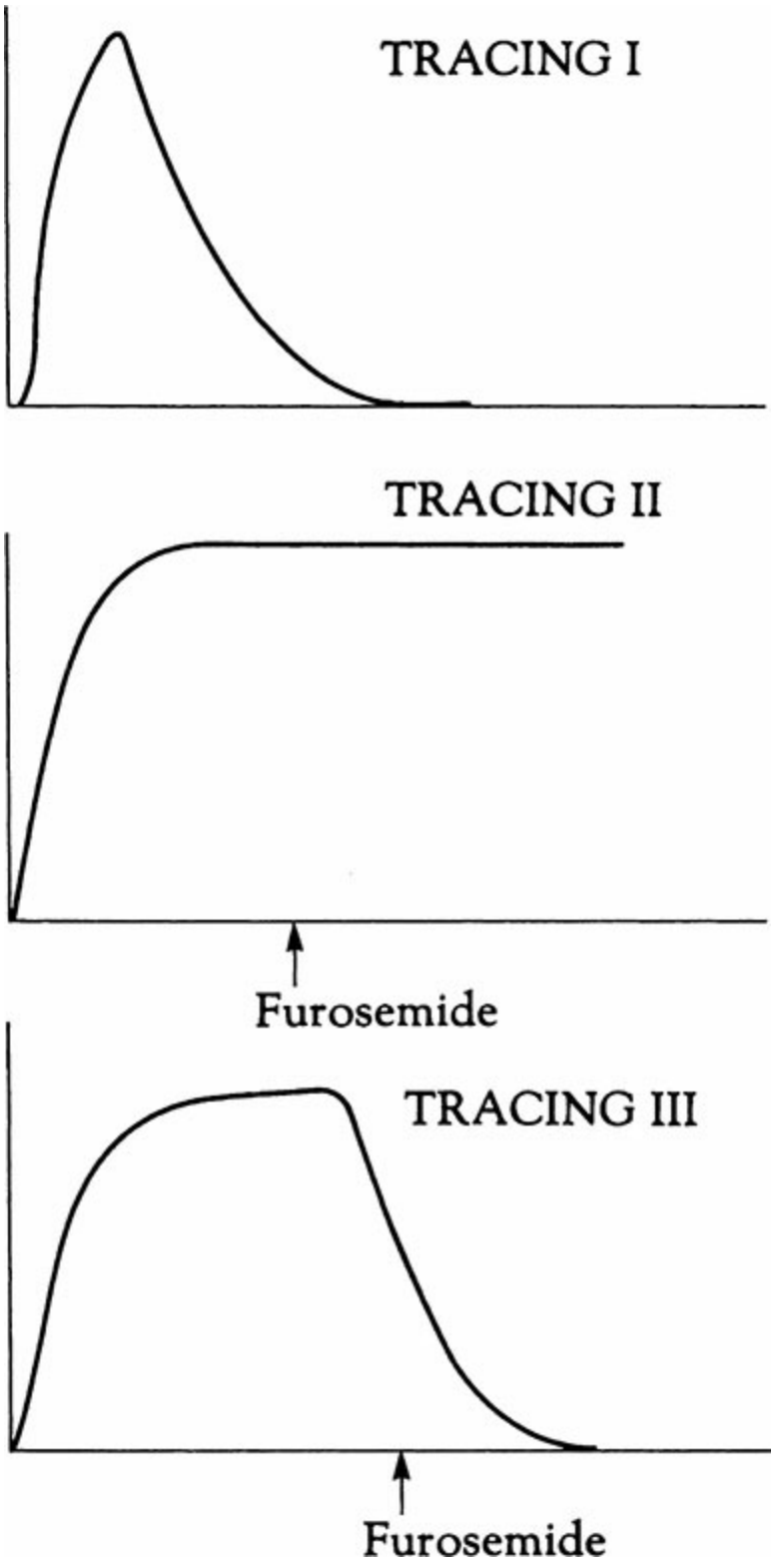

Figure 12–10 Pattern of isotopic renography. Tracing 1, normal excretory patter;

tracing II, obstruction of the urinary tract; tracing III, stasis of urine with obstruction. (Republished with permission of Elsevier Inc, from Gonzalez R, Chiou RK. The diagnosis of upper urinary tract obstruction in children: comparison of diuresis renography and pressure flow studies. *J Urol*. 1985;133:646–649; permission conveyed through Copyright Clearance Center, Inc.)

Pressure-Flow Studies (Whitaker Test)

Pressure-flow studies may be helpful when upper urinary tract obstruction is difficult to diagnose (109), although with modern imaging it is now rarely required. The collecting system is punctured with a fine-gauge needle, and the bladder is catheterized. Fluid is perfused at a rate of 10 mL/minute. At this perfusion rate, the differential pressure between the bladder and the collecting system should not exceed 15 cm H_2O. A differential pressure >20 cm H_2O indicates obstruction, and a pressure gradient between 15 and 20 cm H_2O is equivocal. The pressure-flow study should be done both with an empty and with a full bladder because sometimes the obstruction is only evident when the bladder is full. An antegrade pyeloureterogram can be performed during pressure-flow studies to define the site of any obstruction, eliminating the need for retrograde pyelography.

Additional Tests to Evaluate Lower Urinary Tract Obstruction

A voiding cystourethrogram can be used to investigate the presence of VUR as a cause of the dilatation of the urinary tract.

Obstruction of the lower urinary tract may be evaluated by urodynamic studies and cystoscopy. *Cystoscopy* allows visual inspection of the entire urethra and bladder and can usually be carried out under local anesthetic in adults.

Urodynamic tests measure the urine flow rate per unit of time (debimetry) which depends on the expulsive force of the detrusor muscle and urethral resistance. The patient voids into a container that has a sensor connected to a recorder that plots micturition time and urine flow rate (110). From this plot, the urine volume, duration of micturition, average urine flow rate, maximum urine flow, and time required to reach the maximum flow rate can be calculated and compared to normal values (111). The maximum flow rate is useful in assessing bladder outlet obstruction, but the pattern (continuous or intermittent) of flow also is useful. Physiologic filling of the bladder makes this test more reliable.

Residual urine may be measured after voiding. A normal flow rate with no significant postmicturition urine volume excludes significant bladder outflow obstruction. Cystometry remains the gold standard in differentiating obstructed from nonobstructed men with lower urinary tract symptoms (LUTS) but a number of newer techniques are under evaluation (112).

Treatment of Urinary Tract Obstruction

GENERAL CONSIDERATIONS

Despite the detailed understanding of the pathophysiologic changes that follow ureteral obstruction, the best treatment to hasten the recovery of renal function and limit permanent renal damage remains the prompt and effective relief of the obstruction.

Treatment is dictated by the location of the obstruction, the underlying cause, and the degree of any renal impairment. If renal impairment is present, the treatment of obstruction requires close collaboration between nephrologists and urologists in order to reduce the risks associated with the metabolic and electrolyte consequences of renal failure and to optimize the chances for long-term recovery of renal function. For example, complete bilateral ureteral obstruction presenting as AKI is a medical emergency and requires rapid intervention to salvage renal function. Prompt intervention to relieve the obstruction should result in a rapid improvement in renal function. Dialysis should rarely be required in a patient with AKI secondary to obstruction unless treatment of life-threatening hyperkalemia or severe fluid overload is needed to get the patient fit for intervention. The rapid relief of obstruction will limit permanent renal damage, but renal function may not recover immediately if acute tubular necrosis has occurred as a result of obstruction or any accompanying sepsis.

Surgical intervention can be delayed in patients with low-grade acute obstruction or partial chronic obstruction. However, prompt relief of partial obstruction is indicated when (a) the patient has significant symptoms (flank pain, dysuria, voiding dysfunction), (b) there is urinary retention, (c) there are multiple repeated episodes of urinary tract infection, and (d) there is evidence of progressive renal damage.

MANAGEMENT OF UPPER URINARY TRACT

OBSTRUCTION FROM CALCULI

Calculi are a common cause of ureteral obstruction. Their treatment includes relief of pain, elimination of obstruction, and treatment of infection (113). Pain can be relieved by intramuscular injection of a nonsteroidal antiinflammatory drug, which may help dilate the ureter and aid passage of the stone. In some cases a narcotic analgesic may be needed. High fluid intake to increase urine volume to at least 1.5 to 2.0 L daily may also help mobilize the stone. If possible, any calculi should be recovered for analysis by straining the urine through a gauze sponge or sieve. If the stone is small, the obstruction is partial, there is no infection, and the pain is controlled, expectant management should be followed as many stones will pass spontaneously. Subsequent investigation should be performed to look for metabolic causes for recurrent stone formation and treatment directed accordingly (114).

Intervention may be required for stones larger than 7 mm, since these usually are not passed spontaneously, or if there is persistent colic, urinary tract infection, complete obstruction, or when the calculus has not moved despite an adequate period of observation and increased fluid intake.

There has been a great expansion in minimally invasive techniques for stone removal and open surgery is now rarely required. Options include:

a. Retrograde endoscopic removal using a variety of loops or baskets. This is particularly suitable for calculi located distal to the pelvic brim. This procedure is successful in about 70% of patients. If it fails, dilatation of the ureter or ultrasonic disintegration of the stone can be accomplished using the ureterorenoscope.
b. Percutaneous nephrolithotomy (PCNL), where a nephrostome is placed and the track dilated to provide a direct conduit to the kidney for removal of obstructing pelvic and upper ureteral stones. Rigid or flexible endoscopes can be introduced through the nephrostomy tract to remove calculi <1.5 cm in diameter. For larger stones, lithotripter probes that use ultrasonic or electrohydraulic energy to disintegrate calculi have been used under direct visualization. Endourologic methods can be used to treat obstructing stones successfully in about 98% of patients, shortening the hospital stay and the convalescence period.

ESWL which disintegrates stones in the kidney and upper urinary tract through the use of shock waves has been introduced as an alternative approach. ESWL was first used in the United States in the 1980s and has

had a dramatic effect on the treatment of urinary calculi (115). It involves the focusing of electrohydraulically or ultrasonically generated shock waves to disintegrate the stone into fragments that then can be easily passed. A ureteral JJ stent is frequently placed to allow the stone fragment to pass more easily and painlessly. The treatment is effective for calculi of 7 to 20 mm, and in 90% of patients, the stone is disintegrated and all particulate matter passes within a 3-month period. It works best for stones located within the kidney and is less successful with ureteric stones. Morbidity is low, and the procedure can be done on an outpatient with rapid return to work. Complications include pain, hematuria, and intrarenal and subcapsular hematoma. Hypertension and worsening CKD have been described, and this may be more common in the elderly with preexisting CKD (116). Damage to surrounding organs is rare. A recent Cochrane review indicated that ESWL reduced hospital stay and had a similar efficacy for the treatment of kidney stones to retrograde endoscopic techniques but appeared less effective for kidney stones than PCNL (117). ESWL should not be performed in the presence of urinary tract sepsis.

When obstruction is associated with sepsis, prompt and aggressive treatment is required to prevent life-threatening complications. For antimicrobial therapy to be effective, relief of obstruction is required. The choice of antibiotic should be refined once the results of appropriate urine culture results and sensitivities are known.

MANAGEMENT OF UPPER URINARY TRACT OBSTRUCTION FROM OTHER SPECIFIC CAUSES

A detailed description of all possible surgical treatments of obstructive uropathy is beyond the scope of this chapter. However, the following conditions are commonly encountered in nephrology practice and may be jointly managed with urologists and interventional radiologists:

a. *Idiopathic retroperitoneal fibrosis*. In this condition, ureterolysis (in which the ureters are surgically freed from their fibrous encasement) may be beneficial, especially if combined with steroid therapy to prevent recurrence. A retrospective study has demonstrated the effectiveness of ureteric stent insertion and steroids in idiopathic retroperitoneal fibrosis (118).
b. *Functionally significant PUJ obstruction*. This should be corrected surgically by either an open (Anderson–Hynes pyeloplasty) or laparoscopic approach. The latter results in significantly less morbidity

and has good long-term outcomes that are identical to those of the open procedure. Balloon dilation of the abnormal segment of ureter is also possible, but the recurrence rate is high.

c. *Obstruction secondary to neoplastic, inflammatory, or neurologic disease*. This is unlikely to resolve spontaneously, and some form of urinary diversion such as an ileal conduit may be required. Some obstructing neoplastic lesions, such as lymphadenopathy from lymphoma, may respond to chemotherapy.

NEPHROSTOMY

Nephrostomy is the insertion of a tube through the kidney into the renal pelvis to provide urine drainage (119). It is usually undertaken by interventional radiologists, under local anesthetic and using either ultrasound or CT guidance. Insertion of a nephrostomy is generally the appropriate emergency treatment for upper urinary tract obstruction, especially in the setting of AKI. Insertion should allow rapid recovery of renal function in most patients (>70%), thus avoiding the need for dialysis. Following relief of the obstruction by nephrostomy, the exact site and nature of the obstructing lesion can be determined by infusing X-ray contrast media down the nephrostomy tube (nephrostogram) and time can be taken to plan definitive therapy. Major complications of nephrostomy (abscess, infection, and hematoma) occur in <5% of patients. Bleeding and acute obstruction due to clots may occur, and the tube may become dislodged, requiring immediate replacement.

If both kidneys are obstructed, the nephrostomy should initially be placed in the kidney with the most preserved renal parenchyma, though bilateral nephrostomies may be required to maximize the potential for the recovery of renal function. If infection occurs above a ureteral obstruction (pyonephrosis), then drainage of the kidney with a nephrostomy together with appropriate antibiotics can be lifesaving.

Nephrostomy can be used to gauge the potential for functional recovery in patients with chronic obstruction. Failure of renal recovery after several weeks of nephrostomy drainage strongly suggests irreversible structural damage, and undertaking a more definitive surgical correction of the obstructing lesion is unlikely to be of benefit. Long-term nephrostomy is increasingly used as a definitive therapy for patients who are unsuitable for major surgical intervention and those with incurable malignant disease.

Cystoscopy and passage of a retrograde ureteral catheter may be considered an alternative to nephrostomy to relieve upper urinary tract

obstruction, especially in patients with a bleeding diathesis. However, this may not always be technically possible.

MANAGEMENT OF LOWER URINARY TRACT OBSTRUCTION

Benign prostatic hypertrophy is the most common cause of lower urinary tract obstruction in men and may be mild and nonprogressive. A patient with minimal symptoms, no infection, and a normal upper urinary tract can continue with assessment until he and his physician agree that further treatment is desirable. Medical therapy with either α-adrenergic blockers (e.g., tamsulosin), which relax the smooth muscle of the bladder neck and prostate and decrease urethral pressure and outflow obstruction, or 5α-reductase inhibitors (e.g., finasteride), which inhibit the conversion of testosterone to the active metabolite dihydrotestosterone and reduce prostatic hypertrophy, may be used in patients with moderate symptoms (120). Combination therapy with these agents may be synergistic. Phosphodiesterase-5 inhibitors may also be beneficial particularly in men with coexisting erectile dysfunction (121). With advances in our understanding of the biology of the prostate, it may become possible to adopt an increasing increasingly personalized approach to the treatment of this common condition (122).

Up to 30% of patients do not respond to medical management. Some form of surgical intervention may be required for failed medical treatment, debilitating symptoms, urinary retention, recurrent infection, or evidence of renal parenchymal damage. The gold standard treatment is transurethral resection of the prostate (TURP) but newer techniques are increasingly used. These include holmium laser enucleation of the prostate (HoLEP) which is a less invasive alternative to TURP, with good short-term and long-term outcomes (123) as well as a number of minimally invasive treatment options, which can be performed in an outpatient setting, have a short recovery time, and a good safety profile (124).

Urethral strictures in men can be treated by dilation or direct vision internal urethrotomy. The incidence of bladder neck and urethral obstruction in women is low.

Chronic nonobstructive urinary retention can occur in the absence of a physical obstruction to urine flow, such as in neurologic disorders or from idiopathic causes such as Fowler’s syndrome. This may result in complications such as recurrent urinary tract infections and CKD. The efficacy of treatments such as urethral dilation for nonobstructive urinary

retention is limited and most patients will need to undertake clean intermittent self-catheterization or have an indwelling catheter.

In patients with acute retention of urine, introduction of a urethral catheter will relieve symptoms and allow renal function to recover while definitive therapy is planned. If a catheter cannot be passed, a suprapubic cystostomy may be necessary.

Dynamic studies are essential to determine therapy when obstruction is the result of neuropathic bladder function. The main goals of therapy should be (a) to establish the bladder as a urine storage organ, while preventing renal parenchymal injury and (b) to provide a mechanism for bladder emptying that is acceptable to the patient. Two groups of patients are seen: those with atonic bladders secondary to lower motor neuron injury and those with unstable bladder function owing to upper motor neuron disease. In both cases, ureteral reflux and parenchymal damage may develop, although this is more common in patients with a hypertonic bladder. A neurogenic bladder in diabetes mellitus usually is caused by lower motor neuron disease. Voiding at regular intervals achieves satisfactory emptying of the bladder in these patients. The best treatment for patients with significant residual urine and recurrent bouts of urosepsis is the establishment of clean, intermittent self-catheterization at regular intervals. The goal should be to catheterize four to five times per day such that the amount of urine drained from the bladder does not exceed 400 mL. This technique requires patient acceptance and adequate training.

In patients with hypertonic bladder function, the major goal is to improve the storage function of the bladder. The use of anticholinergic agents (e.g., oxybutynin) may be indicated. Occasionally, chronic, clean, intermittent self-catheterization is necessary.

Chronic indwelling catheters should be avoided, if possible, in all patients with neurogenic bladders. Indwelling catheters can lead to the formation of bladder stones, urosepsis, urethral erosion, and squamous cell carcinoma of the bladder.

MANAGEMENT OF POSTOBSTRUCTIVE DIURESIS

Postobstructive diuresis refers to the marked polyuria that can occur after relief of obstructive uropathy (40). This polyuria is characterized by the excretion of large amounts of sodium, potassium, magnesium, and other solutes. Patients are unresponsive to the administration of ADH.

Although self-limited in duration, the losses of salt and water may result in hypokalemia, hyponatremia or hypernatremia, hypomagnesemia,

and/or marked contraction of the ECF volume and peripheral vascular collapse. In those patients who have had complete obstruction to the urinary tract, a brisk diuresis after relief of obstruction may represent a physiologic response to expansion of the ECF volume occurring during the period of obstruction. This postobstructive diuresis is "appropriate" and does not compromise the volume status of the patient.

Fluid replacement in patients with postobstructive diuresis should be guided by what is excreted. Intravenous and oral fluid replacement is usually required with careful and regular assessment of the patient's fluid balance and serum electrolytes to tailor the fluid replacement regime appropriately. Once the patient is euvolemic, urine losses plus an allowance for insensible losses should be replaced. Urine volume should be measured regularly (hourly), and serum electrolytes should be measured at least daily and as frequently as every 6 hours when there is a massive diuresis. Weighing the patient daily is also helpful. Replacement fluid regimens should include sodium chloride, a source of bicarbonate and potassium. Calcium, phosphate, and magnesium replacement may also be necessary. Orthostatic hypotension and tachycardia are good indicators of when a greater rate of intravenous fluid is required.

If fluid administration is overzealous, the kidney will not recover its concentrating ability and a continued "driven" diuresis will result. It may be necessary to decrease fluid replacement to levels below those of urine output plus insensible losses and observe the patient carefully for signs of volume depletion to distinguish between a driven diuresis and appropriate excretion of excess fluid.

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CHAPTER 13

Renal Physiology and Pathophysiology in Pregnancy

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Normal pregnancy is characterized by alterations in renal and cardiovascular functions that accommodate the hemodynamic and metabolic demands of the growing fetus. Glomerular filtration rate (GFR) and renal blood flow increase in the first trimester, coincident with the dramatic increases in pregnancy-related hormones. Generalized vasodilation is present, and in normal gestation, maternal blood pressure (BP) decreases and cardiac output (CO) increases. These physiologic adjustments are necessary for normal fetal growth and well-being. Reduced GFR and hypertension are risk factors for poor pregnancy outcomes, including fetal growth restriction, preeclampsia, and fetal death. In this chapter, we will discuss the fundamentals of renal physiologic principles in pregnancy, the unique considerations relevant to pregnancy in women with kidney disease, and the hypertensive disorders in pregnancy.

Renal Physiology in Pregnancy

MATERNAL CIRCULATION AND BLOOD VOLUME REGULATION IN PREGNANCY

During normal pregnancy, the maternal circulation is characterized by

vasodilation, which starts in the sixth week of gestation and leads to a decrease in systemic vascular resistance (SVR) (1,2). Vasodilation has been attributed to an increase in progesterone, prostacyclin, vascular endothelial growth factor (VEGF) (3), nitric oxide (4–9), angiotensin 1–7 (10,11), and relaxin (12–20). To date, the exact mechanisms leading to systemic vasodilation and the degree to which each of these factors contributes have not been completely defined.

In order to maintain adequate blood volume despite a decreased SVR, maternal total body water is increased by 6 to 8 L, with an increase of 4 to 6 L in the extracellular compartment (21). Renal plasma flow (RPF) increases early in pregnancy also by the sixth week of gestation. By midpregnancy, the plasma volume is increased by 50% (21,22), leading to a 40% to 65% rise in RPF (as measured by para-aminohippurate clearance) (23). RPF peaks at 80% above prepregnancy levels during the second trimester (21,22) and declines in the third trimester to 60% greater than prepregnancy values (23).

There is also a net reabsorption of 500 to 950 mEq of sodium during pregnancy (21,22), which permits maternal plasma volume expansion. Sodium homeostasis is discussed in further detail later in this chapter. Pregnancy-associated volume expansion resolves rapidly by 2 weeks postpartum (24).

Plasma volume expansion in pregnancy is well tolerated, although peripheral edema is not uncommon. It is typically an adaptive phenomenon rather than a pathologic state of volume overload and does not adversely affect cardiovascular hemodynamics. During pregnancy, SVR drops, whereas CO, stroke volume, and heart rate are all increased (25–29). CO increases by 11% during the first trimester, remains stable during the second trimester, and increases again to 17% after 28 weeks of gestation (25), with the highest CO reported being 50% greater than nonpregnancy values (26,28,29). The timing of the CO peak and the magnitude of the increase in the third trimester have diverged across studies (29,30) potentially related to differences in methodologies where echocardiography and impedance have both been performed. Postpartum, CO returns to prepregnancy levels in approximately 2 weeks (31). Measurements of CO were done by echocardiography in 74% of studies and by impedance for 18% (29).

BLOOD PRESSURE REGULATION IN NORMAL PREGNANCY

During pregnancy, generalized vasodilation leads to a decrease in mean arterial blood pressure (MAP) of approximately 10 mm Hg (32), starting early in the first trimester, with a nadir between 12 and 28 weeks of gestation (2,32,33). In late pregnancy, BP gradually starts to increase again back to prepregnancy levels. Normal BP in pregnancy is lower compared to nonpregnancy; however, optimum BP has not been defined. Women are considered to have hypertension if BP is greater than or equal to 140/90 mm Hg. However, this threshold does not adequately address what is the optimum BP for most women.

All components of the renin–angiotensin–aldosterone system (RAAS) are increased during pregnancy. Plasma renin activity (PRA) rises by 6 weeks of gestation (2) and may quadruple by midpregnancy (32). The early gestational rise in PRA is likely a consequence of vasodilation and lower BP, and is accompanied by a rise in aldosterone (2). Blockade of the RAAS with a single dose of captopril, administered to first and second trimester pregnant women, results in an augmented drop in BP and rise in renin compared to nonpregnant women (34), suggesting that the stimulated RAAS in pregnancy is a physiologically appropriate response to lower BP. Chapman et al. reported that in the third trimester, aldosterone continues to rise, whereas PRA remains constant (2). We and others have found that both serum and urine aldosterone concentration continuously increase as pregnancy progresses (35–37). Our results also suggest that PRA and aldosterone levels are highly correlated throughout gestation (unpublished results). The recent studies of Gennari-Moser et al. (38) demonstrate that elevated aldosterone levels in pregnancy help maintain BP and are also consistent with the notion that the stimulated RAAS in pregnancy is a physiologic response to the hormonally mediated changes in BP and vasodilation.

Several investigators have observed that the maternal vasculature is significantly less responsive to angiotensin II, norepinephrine, and vasopressin (as compared to nonpregnant women) (39–46). The basis for this observation is not known; it may be a consequence of "downregulation" of vascular receptors resulting from higher levels of circulating angiotensin II occupying and internalizing these receptors, or it may reflect the fact that BP is lower owing to vasodilatory pregnancy-related hormones that lead to compensatory increases in angiotensin II, norepinephrine, and other pressors that do not generate as significant a BP response.

Gestational resistance to the stimulated RAAS is relative, and the RAAS remains an important regulator of salt balance and BP in pregnancy

(47). The RAAS demonstrates typical endocrine regulation, and aldosterone increases after standing up in pregnancy (48,49) and decreases with salt loading (35,49). As mentioned, in women admitted for pregnancy termination, the administration of an angiotensin-converting enzyme inhibitor leads to a marked hypotensive response and increase in PRA (34). Salt loading in pregnant women results in similar changes in urine Na levels as seen in nonpregnant women (38).

The interaction of estrogen, progesterone, and the RAAS, and their role in BP regulation in pregnancy is particularly intriguing. Elevations of estrogen are in part responsible for the elevations in angiotensinogen in pregnancy and lead to a doubling of PRA (32,50,51). Progesterone is a weak mineralocorticoid receptor antagonist (52,53), which leads to aldosterone resistance by competitively binding to the mineralocorticoid receptor (54,55). Progesterone may also decrease BP by other natriuretic mechanisms (56), including impairment of proximal Na reabsorption (52).

The sympathetic nervous system has been reported to be overly active during the first few weeks of pregnancy (57) as well as during the third trimester (58). Some investigations suggest that sympathetic nervous system overactivation is even more pronounced in hypertensive pregnancies; however, the impact on sustained hypertension is unclear (58–61).

RENAL ANATOMY DURING NORMAL PREGNANCY

There is a 30% increase in kidney size during pregnancy (62,63), most likely a result of the increased renal blood flow and resulting vascular and interstitial expansion. Relaxation and expansion of the renal calyces is also reported as a physiologic finding (62,64–68). This has been attributed to compression of the ureters between the iliopsoas muscle and the gravid uterus (69–71). However, the appearance of dilatation of the urinary tract on renal ultrasound may also be a consequence of hydronephrosis, especially in the third trimester (72,73). These anatomic alterations may contribute to the development of bacteriuria and pyelonephritis in pregnancy.

RENAL FUNCTION IN PREGNANCY

Glomerular Hemodynamics in Pregnancy

In the context of generalized vasodilation and volume expansion, it is not

surprising that the GFR is increased during pregnancy (8,23,24,48,74–80) (Fig. 13-1). Inulin-measured GFR and creatinine clearance increase by 50% early in the first trimester (23,81). By the third trimester, increases of up to 75% above nonpregnant levels are reported (2). As previously mentioned, RPF rises even more (by up to 80%) in early pregnancy (23,74–76) and returns to prepregnancy levels by the third trimester (2,23). RPF remains stable and even slightly lower than prepregnancy levels in the postpartum period (82). The filtration fraction, defined as the ratio of GFR/RPF, is therefore lowered earlier in pregnancy (from 6 to 36 weeks of gestation) (2) and subsequently increases (2,75,82). Although volume expansion resolves by 2 weeks after delivery, the GFR remains 20% above prepregnancy levels (24) and only returns to prepregnancy levels a month postpartum (83) (Fig. 13-2).

It is likely that there are mechanisms in addition to vasodilation and increased RPF underlying the increase in GFR during pregnancy. Older data suggest that progesterone may contribute to the gestational increase in GFR (55). Relaxin has been associated with gestational vasodilation but is not likely to be the primary mediator of the renal hemodynamic changes and GFR increase during human pregnancy (18,84). Other factors believed to be contributing to the gestational rise in GFR include a decrease in vascular and glomerular oncotic pressure, and increased glomerular basement membrane permeability noted in pregnancy (77,85,86) and also in the postpartum period (24,82). A potential role for estrogen in the increased GFR in pregnancy has been postulated, based in part on gender differences in chronic kidney disease (CKD) progression demonstrating better outcomes in women compared to men, and this is an area worthy of further study (87).

Consistent with increases in measured GFR, creatinine clearance also increases progressively during pregnancy (81). The 24-hour urinary creatinine excretion, however, remains unchanged (2), leading to an overall decrease in serum creatinine during pregnancy from an average of 0.83 mg/dL (73 *m*mol/L) prepregnancy to 0.74 mg/dL (65 *m*mol/L) in the first trimester, 0.58 mg/dL (51 *m*mol/L) in the second trimester, and 0.53 mg/dL (46 *m*mol/L) in the third trimester (88). This decrease in serum creatinine is not solely attributable to an increase in GFR but may also be influenced by an increase in maternal nonmuscle body weight. Creatinine-based formulas such as the Modification of Diet in Renal Disease (MDRD) (89–91), Cockroft–Gault (91,92), or Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (93) are frequently used to estimate GFR but have not been validated in pregnancy and are not

thought to be accurate calculations of GFR in this setting. Cystatin C-based formulas to estimate GFR have also not been validated in pregnancy (91,94). Consequently, the best estimator of GFR during pregnancy remains creatinine clearance (determined by a 24-hour urine collection).

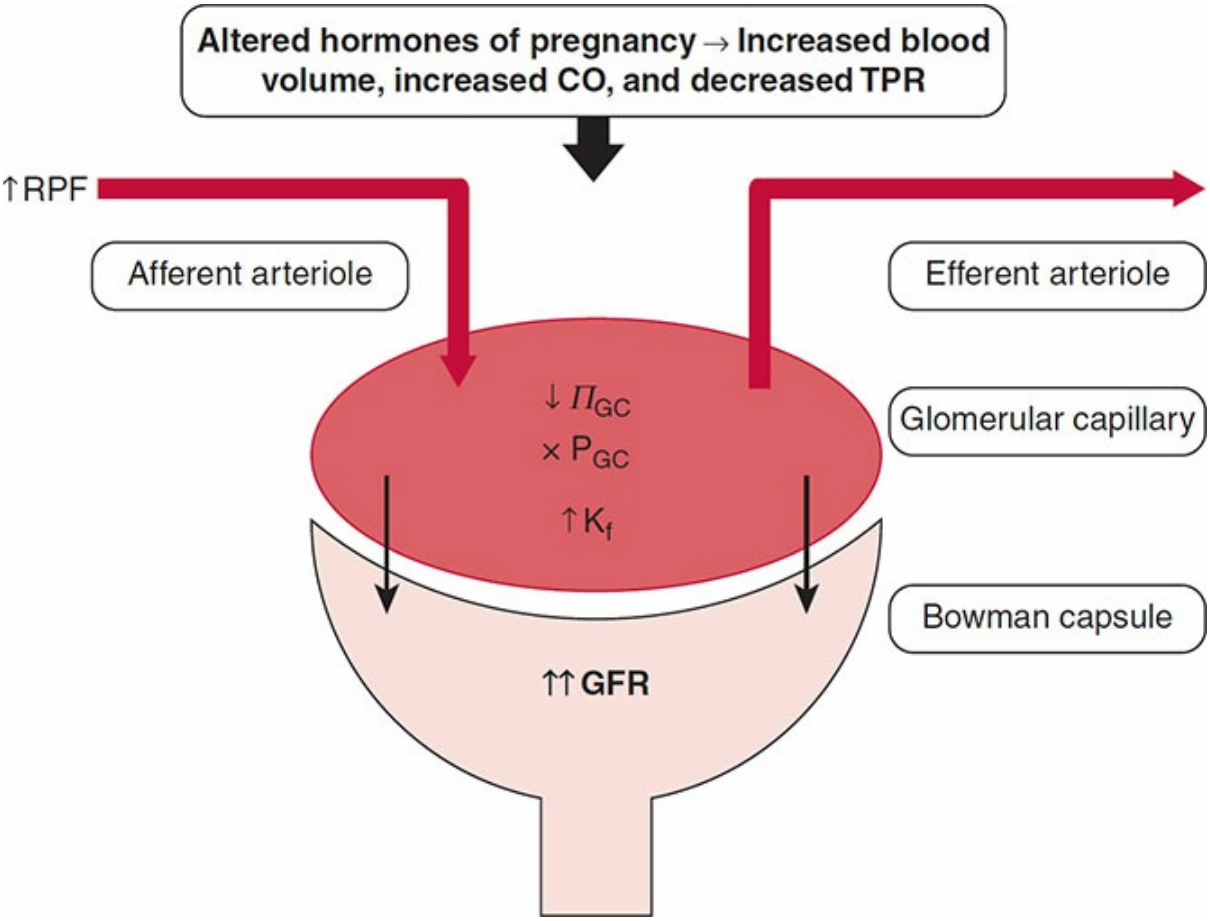

Figure 13–1 Renal hemodynamic changes in pregnancy. Black arrows depict changes in comparison with the nonpregnant state. CO, cardiac output; TPR, total peripheral resistance; RPF, renal plasma flow; P_{GC}, glomerular oncotic pressure; P_{GC}, glomerular capillary pressure; K_f, transcapillary ultrafiltration coefficient; GFR, glomerular filtration rate. (From Hussein W, Lafayette RA. Renal function in normal and disordered pregnancy. *Curr Opin Nephrol Hypertens*. 2014;23(1):46–53, with permission from Wolters Kluwer Health, Inc.)

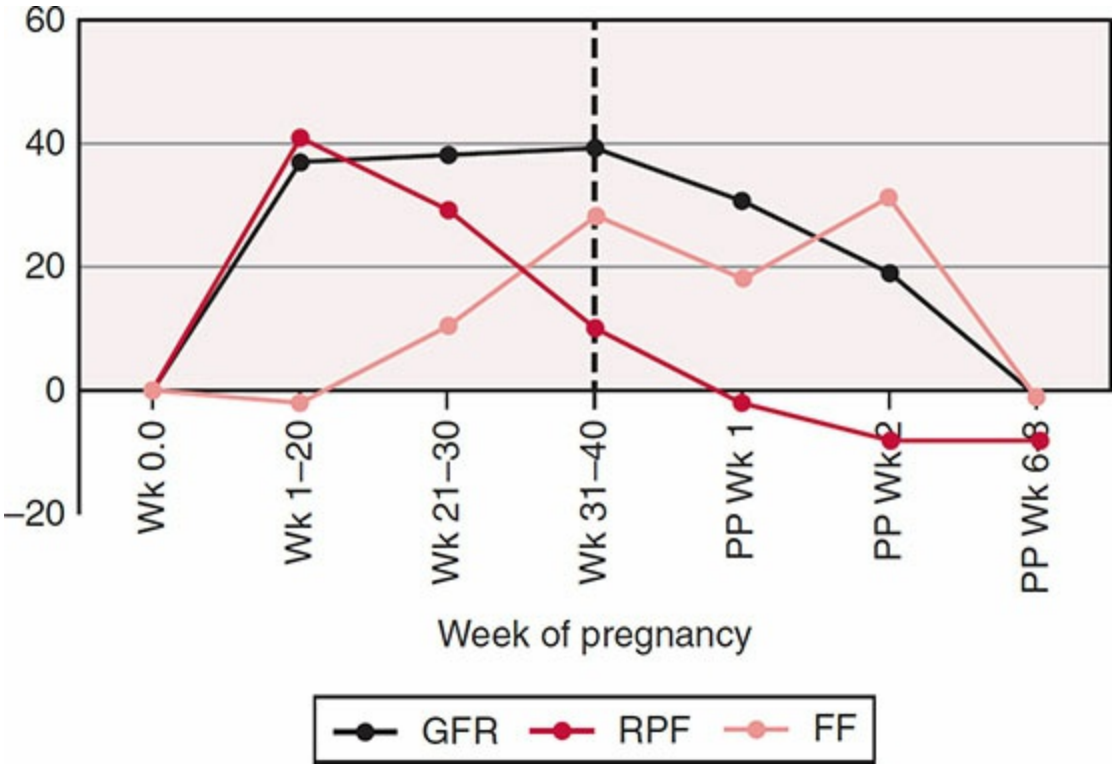

Figure 13–2 Longitudinal changes in renal hemodynamic parameters during pregnancy. The percentage change in GFR, RPF, and FF are expressed at different time points in gestation. GFR was measured by inulin or iothalamate and RPF by *p*-aminohippurate clearance methodology, respectively, at different time points during gestation. FF, filtration fraction; GFR, glomerular filtration rate; RPF, renal perfusion flow. (Republished with permission of American Society of Nephrology from Odutayo A, Hladunewich M. Obstetric nephrology: renal hemodynamic and metabolic physiology in normal pregnancy. *Clin J Am Soc Nephrol*. 2012;7(12):2073–2080; permission conveyed through Copyright Clearance Center, Inc.)

TUBULAR FUNCTION IN PREGNANCY

During pregnancy, GFR and RPF increases lead to increased demands on tubular transport. Tubular reabsorption may fail to match these rising demands, leading to increased excretion of protein, glucose, and amino acids (95).

Proteinuria and Albuminuria in Normal Pregnancy

Normal pregnancy is associated with an increase in daily urinary protein excretion (96–99). Proteinuria is normally below 150 mg/day; it increases by approximately 100 mg/day during pregnancy (97) and may reach 260 mg/day in normal pregnant women (99). Increased proteinuria is more common in twin compared to singleton gestations (100). The threshold for

abnormally elevated proteinuria is considered to be greater than 300 mg/day during pregnancy (101).

During pregnancy, the total daily albuminuria is not significantly increased (97,102–106) and therefore does not account for the physiologic proteinuria of normal pregnancy. A few studies have reported that the urinary albumin to creatinine ratio is elevated in pregnancy (107) and increases as gestation progresses (108,109) and in the peripartum period (102). Despite these observations, the urinary albumin to creatinine ratio does not increase enough to account for the important increase in proteinuria (107) and does not reflect an overall increase in the 24-hour excretion of albumin (106).

The preponderance of nonalbumin proteinuria therefore points to a tubular rather than glomerular origin of proteinuria. Indeed, tubular proteins such as retinol-binding protein (97,104,110), *N*-acetyl-β-glucosaminidase (98,104,107,111–114), β_2-microglobulin (104,107,115), α_1-microglobulin (104), transferrin (98,115), alanine aminopeptidase (98,114), and Clara cell protein (104) are elevated in the urine of healthy pregnant women. Whether this is a manifestation of tubular dysfunction and impaired tubular protein reabsorption is not clear.

Uric Acid Excretion in Normal Pregnancy

Increased GFR and decreased tubular reabsorption during pregnancy lead to an increase in urinary excretion of uric acid and generally lower levels in plasma and serum (56,95). As pregnancy progresses (116) beyond 20 weeks (117), uric acid excretion decreases and plasma levels of uric acid may increase again to prepregnancy levels in the third trimester (116,118).

Glucose and Amino Acid Excretion in Normal Pregnancy

Glucosuria may be present in normal pregnancies, and older studies suggest that it is because of decreased proximal tubular reabsorption (119–121). Proximal tubular glucose reabsorption may be decreased in pregnancy owing to volume expansion, leading to decreased sodium reabsorption and therefore less glucose reabsorption (122,123). Increased GFR and hyperfiltration, as well as glucose loading, may further saturate the ability of the tubules to reabsorb glucose and induce glucosuria (122,124) independently of glycemia (125). Therefore, mild glucosuria is not a reliable screening test for diabetes in pregnancy (126,127).

Amino acids are also reabsorbed by the proximal tubule and are

excreted in the urine in larger amounts during pregnancy (125). Different amino acids have distinct patterns of variation throughout gestation (128). Water-soluble vitamins, including nicotinic acid (129), vitamin C (125), and folate (125,130,131), are also found in larger amounts in the urine of pregnant women.

WATER HANDLING IN PREGNANCY

During pregnancy, plasma osmolality is decreased by 5 to 10 mOsm/kg early in the first trimester with a decrease in serum sodium by 5 mEq/L. Pregnancy is considered to be a condition characterized by a reset osmostat, because osmolality is maintained at this level with appropriate osmoregulation in response to water excess and deprivation (132–136). There is also evidence of nonosmotic release of antidiuretic hormone (ADH) in the setting of vasodilation and lower BP (137).

Measurement of serum ADH or vasopressin is technically difficult given that circulating levels are typically low and below the level of detection of the assay; therefore, investigators have recently started to measure copeptin as a surrogate for ADH. Copeptin is co-secreted with ADH and has a longer half-life, and the assay allows for detections of relatively low circulating levels, which renders this peptide easier to measure reliably (138,139). During pregnancy, copeptin levels rise with gestational age (139); this occurs to a larger extent if preeclampsia develops (138,139).

Leucine aminopeptidase (140), also referred to as placental vasopressinase (141), has been described as an important mitigator of physiologic hyponatremia of pregnancy. In certain instances, in the third trimester, placental vasopressinases may cause ADH resistance that is manifested as gestational diabetes insipidus. Because these enzymes act at the *N*-terminal of the native ADH molecule, administering dDAVP (a synthetic agonist of ADH with a different *N*-terminal) may correct gestational diabetes insipidus (142).

Potential mechanisms for a reset osmostat in pregnancy include elevated levels of β-human chorionic gonadotropin (HCG) (134,143) and relaxin (144,145). It has been hypothesized (136) that β-HCG induces relaxin release from the placenta, which then causes the reset osmostat of pregnancy; however, this has not been reproduced experimentally.

ELECTROLYTE HANDLING IN PREGNANCY

Calcium and Vitamin D Balance

Both total serum calcium (Ca) and albumin decrease in pregnancy; however, ionized Ca remains unchanged (146,147). An increase in Ca clearance and urinary Ca excretion has been reported, but the fractional excretion of ionized Ca is relatively stable in pregnancy (147). The increase in urinary total Ca excretion may be attributed to an increase in GFR (147) and to an increased dietary Ca intake that is encouraged by many obstetricians in order to maintain adequate calcium balance for fetal bone mineralization (148). Also, 25-hydroxylation of vitamin D is increased during normal pregnancy (149) owing to placental conversion of vitamin D_3, resulting in levels of 1,25-dihydroxyvitamin D (1,25-D) that are two to three times the nonpregnant levels and result in increased intestinal calcium absorption (reviewed by Hojo and August (150)). Calcitonin increases (151) and intact PTH (parathyroid hormone) decreases in order to counteract bone resorption (152).

Calcium metabolism is dysregulated in women with preeclampsia. Decreased urinary excretion of calcium has been reported both prior to and at the time of diagnosis of preeclampsia (153–157). The mechanism of hypocalciuria may be in part related to the mild decreases in GFR; however, changes in both 1,25-D and PTH may also be responsible (153,158). 1,25-D is lower in women with preeclampsia (155,159–161), and women with lower levels of 25-hydroxyvitamin D (less than 30 nmol/L as opposed to 50 or above, at 14 weeks) (162) and 1,25-D (159) are at a higher risk of preeclampsia, preterm birth, and small for gestational age (SGA) infants (163). Several clinical trials have suggested a benefit of calcium supplementation in prevention of preeclampsia (164). Although not extensively studied, there is some suggestion that vitamin D supplementation may have beneficial effects with respect to prevention of hypertension in pregnancy (149,165). Despite hypocalciuria and decreased levels of active vitamin D, ionized calcium is maintained at a normal or mildly lower level in preeclampsia. Intact PTH is reported to be increased in preeclampsia in some, but not all, studies (155,159,160).

Sodium and Potassium Homeostasis

Total body sodium (Na) and total body potassium (K) both increase during pregnancy in order to accommodate the developing fetus and placenta. Na accumulation is estimated to be 900 to 1,000 mEq (approximately 2–6 mEq/day). Total potassium accumulation is approximately 320 mEq (86).

Total body electrolyte stores are not necessarily reflected by serum electrolyte concentrations because of the increased plasma volume. Normal ranges for electrolytes have not been clearly established in pregnancy despite the observation that serum/plasma Na and K are often lower in the first two trimesters of pregnancy (166–168), owing to both hemodilution and hypoosmolality of pregnancy.

Renal Na and K excretion in pregnancy have not been studied extensively. Urinary Na excretion (U_{Na}) decreases with gestational age (37,169). However, the literature is not unanimous about the patterns of urinary K excretion (U_K). Tamas et al. report an increase in U_K (37), whereas Ehrlich and Lindheimer report that on a controlled diet, U_K is stable throughout pregnancy (170). In both cases, as the pregnancy proceeds, the ratio of U_{Na} to U_K drops (37,170), and the administration of a mineralocorticoid affects U_{Na} but not U_K (170). This uncoupling of Na and K excretion furthers the suspicion that Na and K excretions are differentially regulated by aldosterone in normal pregnancy or that other nonmineralocorticoid pathways play a significant role. Na retention occurs in synchrony with increasing aldosterone (167). A study by Tamas et al. failed to demonstrate a correlation between urine aldosterone and U_{Na} (37).

The role of progesterone in the regulation of U_{Na}, U_K, and aldosterone is of considerable interest, but has not been extensively studied (86,170). Progesterone antagonizes aldosterone's binding to the mineralocorticoid receptor, thereby preventing Na reabsorption by principal cells (54,171) (refer to BP Regulation in Pregnancy section) and may also prevent K excretion (170). In this regard, the observations that, in some women with primary hyperaldosteronism, both BP and serum K levels are normalized during gestation are of interest, and are consistent with an antimineralocorticoid effect of progesterone (172). During pregnancy, there is an increased extra-adrenal production of deoxycorticosterone, which may further contribute to Na retention (173). Natriuresis may also be increased independently of mineralocorticoid antagonism as a consequence of increased atrial natriuretic peptide levels (2,86,174), and possibly progesterone acting on proximal tubular reabsorption of sodium (52). Given the recent literature emphasizing the importance of the relationship between U_{Na}, U_K, and hypertension (175), additional studies of these electrolytes in hypertensive pregnancies are potentially important. Thus far, most studies of renal Na transporters and channels (including the

Na^+/K^+ ATPase and the epithelial sodium channel) still need to be verified and reproduced in other animal studies.

ACID–BASE BALANCE IN PREGNANCY

During pregnancy, there is increased minute ventilation, alveolar ventilation, and tidal volume, which all lead to a decrease in arterial partial pressure of carbon dioxide (PCO_2) and lower levels of circulating hydrogen ions (176–179). Progesterone is thought to be the stimulus for lowering PCO_2 because progesterone levels are associated with both an increased minute ventilation and a lower PCO_2 (176,180–182). Other potential triggers for increased ventilation in pregnancy include hypoosmolality and elevated ADH (179,182). In response to relative respiratory alkalosis, renal compensation occurs with a decrease in serum bicarbonate to levels in the 18 to 22 mEq/L range (179,183).

Kidney Disease in Pregnancy

Historically, women with CKD were often discouraged from becoming pregnant because of the high rates of maternal and fetal morbidity and mortality. With advances in neonatal intensive care, more sophisticated multidisciplinary maternal care, utilization of erythropoietin-stimulating agents as well as successful assisted reproduction, it is not unusual for women with CKD to become pregnant. These pregnancies are high-risk, and should be managed by a multidisciplinary team including a high-risk obstetrician and a nephrologist or internist. Women with a history of vasculitis and autoimmune disease should be closely monitored for immune activation that may resemble superimposed preeclampsia, whereas women with morphologic renal diseases (urinary tract malformation) are at a higher risk for infection (184). Specialists who are trained to care for pregnant women should be sought for the care of women with high burden of disease. It is also important to pursue birth control options and family planning in any woman of reproductive age with CKD in order to preemptively discuss risks and benefits of pregnancy in the context of the individual patient's psychosocial and medical conditions (185).

CHRONIC KIDNEY DISEASE IN PREGNANCY

Stages of Chronic Kidney Disease and Effect on Pregnancy Outcomes

A diagnosis of CKD doubles the risk for adverse maternal outcomes and for preterm delivery (186,187). Fetal and maternal outcomes including preeclampsia, gestational hypertension, preterm birth, intrauterine growth restriction (IUGR; fetal size below the 10th percentile), SGA infants (birth weight below the 10th percentile), and even death are more common in women with a history of CKD (186,187,189). CKD, independent of its etiology (188) or other comorbidities (187,190), leads to worse pregnancy outcomes.

Even women with a normal GFR or stage 1 CKD are at an increased risk for adverse fetal outcomes (preterm birth, SGA, need for neonatal intensive care), new onset hypertension, new/doubling proteinuria; but not intrauterine fetal demise compared to women without CKD (188,191). Multigravid pregnancies increase fetal risks for an earlier delivery, low birth weight, intensive care needs, and even mortality, and if possible should be avoided in women with CKD (192).

Women with more advanced CKD (stages 4–5) at the start of pregnancy have worse maternal and fetal outcomes than women with milder CKD (stages 1 or 2) (191,193). When GFR is between 60 and 90 mL/kg/1.73 m^2, the odds of developing preeclampsia or worsening renal function are increased by 6.75-fold compared to normal pregnancies, and the odds of developing adverse fetal outcomes (premature birth, IUGR, fetal death, low birth weight) triple (194). In women with an even lower GFR ($<$30 mL/kg/1.73 m^2), prematurity rates increase further and reach 89% (as opposed to 41% with baseline GFR 45–100 mL/kg/1.73 m^2, P = 0.02) (195).

Effect of Pregnancy on Renal Function in Chronic Kidney Disease

A meta-analysis of over 500,000 pregnancies showed that despite higher risk of poor pregnancy outcomes (preeclampsia, preterm birth, or SGA) in women with CKD stages 1 to 3, pregnancy was not clearly associated with long-term worsening in renal function (196). In women with preserved renal function, CKD 1, or mild renal impairment (Cr $<$ 1.4 mg/dL), pregnancy does not seem to lead to long-term worsening of renal function (197,198). The risk profile is different for women who do not have a

preserved renal function (with a Cr $\geq$ 1.4 mg/dL) or who have hypertension (199–202). Women with advanced CKD at baseline, however, are at a higher risk for deterioration in renal function as a result of pregnancy.

A baseline GFR below 40 mL/min/1.73 m^2 significantly increases the risk for further renal function decline in pregnancy (203). In a study of 98 pregnant with CKD, having a GFR $>$ 90 mL/min/1.73 m^2 did not result in worsening in renal function (defined as a 25% increase in creatinine). Nevertheless, 41.3% of women with GFR 60 to 90 mL/min/1.73 m^2 and 60% of those with GFR 15 to 59 mL/min/1.73 m^2 developed worsening in renal function during pregnancy (P = 0.001) (194). As previously discussed, estimation of GFR using creatinine-based formulas may not accurately reflect renal function during pregnancy; thus, serum creatinine levels are recommended for monitoring renal function in these patients.

Katz et al. reported that in 121 women with mild renal insufficiency (serum creatinine level less than 1.4 mg/dL), only 16% had worsening renal function that was reversible in all cases (197). The risk for preterm birth was approximately 20%, with a 24% risk for IUGR and favorable fetal survival rates (89%) (148,197,204–206).

Jones and Hayslett reported that in women with prepregnancy serum creatinine level of 1.4 to 2.5 mg/dL, 50% developed worsening renal function (200), and among women who developed worsening renal function during pregnancy, 23% progressed to dialysis dependency within 6 months of delivery, whereas the rest recovered renal function (200). Older studies report fetal survival rates of 76% to 80% in women with moderate renal insufficiency (207–209). In a small study of 29 pregnant women with CKD, by Sato et al., four of seven women with moderate renal insufficiency were initiated on dialysis during pregnancy for blood urea nitrogen (BUN) $>$100 mg/dL (202).

Women with a prepregnancy serum creatinine above 2.5 mg/dL are at a high risk (40%) for developing dialysis dependency during pregnancy or postpartum. Preeclampsia is also very common in this group and affects at least 40% of pregnancies. Fetal outcomes are poor: 73% preterm birth and 57% IUGR (200). All of the 17 women with a prepregnancy creatinine $>$2.5 mg/dL in Sato et al.'s study were started on dialysis during pregnancy (202). Given the high fetal and maternal morbidity, we do not recommend pregnancy in women with this degree of renal dysfunction. A discussion of other options such as adoption or, when available, surrogacy is advised. In addition to reduced GFR, proteinuria of more than 1 g/day is associated with poor pregnancy outcomes (188,191,203).

The mechanism for worsening renal function in pregnant women with underlying CKD is not fully established. Experimental models have suggested that the etiology of CKD may be an important factor in glomerular response to pregnancy. Baylis et al. performed micropuncture experiments in a variety of experimental models of CKD during pregnancy. In all models that were studied, the glomerular capillary pressure (P_{GC}) was not increased, suggesting that glomerular hypertension is not the primary cause of deterioration in renal function during pregnancy. The lack of increase in P_{GC} is mediated by vasodilation of either the systemic or renal vasculature, depending on the cause of CKD. In the rat model of anti-basement membrane glomerulonephritis in pregnancy, renal vasodilation prevents further increases in P_{GC}. In the 5/6 nephrectomy model, renal vasodilation is associated with a decrease in P_{GC} (210). In the rat model of membranous glomerulopathy in pregnancy, there is decreased systemic BP, reduced P_{GC}, and preferential constriction of the renal afferent arterioles.

There are data suggesting that hypertension is an important risk factor for adverse outcomes in pregnancies in women with CKD (201,211). Masuyama et al. reported that fetal outcomes (gestational age at delivery, need for intensive care unit, SGA, Apgar score) are more closely associated with elevated BP than proteinuria (212).

Hereditary Kidney Disease

Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is the most common form of hereditary kidney disease, and reproductive age women may have hypertension and CKD, increasing their risk for pregnancy complications (213). Subclinical disease is occasionally present at conception, and a diagnosis is occasionally first made during pregnancy. Genetic counseling is recommended in women with a family history of ADPKD.

Women with ADPKD are at increased risk for complications compared to unaffected family members, especially if they have hypertension (213). The risks for adverse fetal events are increased with maternal age $^{>}30$, reduced GFR, hypertension, and superimposed preeclampsia (213). A recent study of 92 pregnancies in women with ADPKD and well-preserved kidney function found an increased risk for hypertension, proteinuria, renal

dysfunction, and preeclampsia compared to a control group of women with simple renal cysts (214). Rates of premature birth or spontaneous abortion were similar to those in the control group (214). The literature also suggests an increased risk of incident and recurrent urinary tract infections (UTIs) (214,215), and we recommend frequent urine cultures during pregnancy.

Repeated pregnancies may adversely affect long-term renal function in women with ADPKD (213) and increase the risk for the development and enlargement of liver cysts (216). Screening for cerebral aneurysms should be considered prior to labor, especially in women with a family history of cerebral aneurysms.

Alport Syndrome

There are only a few reports of pregnancy in women with Alport syndrome, and as in women with other etiologies of CKD, the risks of maternal and fetal complications are increased. In one small series of 18 pregnancies, deterioration in renal function (in 5/18 pregnancies), preterm birth (10/18), and stillbirth (2/18) were common (217). Evidence for the management of Alport syndrome in pregnancy is limited to case reports and small case series which suggest that in women with reduced GFR and hypertension, complications are more common (218,219)

Lupus Nephritis

Lupus nephritis during pregnancy presents unique problems. Although similar considerations apply regarding the relationship between level of renal function and BP to pregnancy outcome, in general, lupus is a much more unpredictable illness, because of the tendency of the disease to flare (220). Increased organ damage following pregnancy in women with lupus is associated with pregnancy duration, total disease duration, and disease activity and damage prior to pregnancy (221). Whether or not pregnancy per se is a risk factor for lupus flares has been disputed. Although some report no increase in flares attributable to pregnancy in patients in remission, prospective data suggest that pregnancy is in fact associated with a greater chance of disease exacerbation (222). Women with lupus nephritis are at greater risk for adverse maternal and fetal outcomes compared to women without renal involvement and are advised not to conceive unless their disease has been “inactive” for the preceding 6 months, because there is a higher incidence of fetal demise when disease is

active at conception. Inactive disease usually means a creatinine measurement of <0.7 mg/dL or 62 *m*mol/L, proteinuria less than 0.5 g/day, and on spun urine examination, fewer than five red blood cells per high powered field. If there is active disease with a creatinine of >1.2 mg/dL or 106 *m*mol/L, fetal loss occurs in 25% to 50% (223).

Additional complications associated with lupus and pregnancy include placental transfer of maternal autoantibodies, which can cause a neonatal lupus syndrome characterized by heart block, transient cutaneous lesions, or both. Women with lupus are also more likely to have clinically significant titers of antiphospholipid antibodies (anticardiolipin antibody or lupus anticoagulant) which are associated with spontaneous fetal loss of 50% to 75%, hypertensive syndromes indistinguishable from preeclampsia, and thrombotic events including deep vein thrombosis, pulmonary embolus, myocardial infarction, and stroke (224). Thus, all women with systemic lupus erythematosus (SLE) should be screened for antiphospholipid antibodies early in gestation. When titers are elevated (more than 40 GPL), low-dose daily aspirin (80–325 mg) is recommended. If there is a history of thrombotic events, then low molecular weight heparin (LMWH) in combination with aspirin is recommended. Therapy with unfractionated or LMWH in addition to aspirin has been advocated for secondary prevention in women with a history of stroke, myocardial infarct, deep vein thrombosis, pulmonary edema, or placental thrombosis (225).

One of the difficulties in managing lupus nephritis during pregnancy is that increased activity of lupus may be difficult to distinguish from preeclampsia. Both are characterized by an increase in proteinuria, a decrease in GFR, and hypertension. Thrombocytopenia may also be observed in both conditions. Hypocomplementemia is not a feature of preeclampsia, whereas increases in liver function tests may be observed in preeclampsia but are not characteristic of lupus activity. If disease activity is present before 20 weeks of gestation, then the diagnosis is more likely to be a lupus flare. In the latter half of pregnancy, it may be impossible to distinguish between a renal lupus flare and preeclampsia. In fact, frequently both are present simultaneously as increased lupus activity often triggers increased BP and proteinuria, which is indistinguishable from preeclampsia. Spun urine microscopy for red blood cell casts can also signal lupus nephritis activity. Biomarkers such as fms-like tyrosine kinase-1 (sFlt1) and placental growth factor (PlGF) may help discriminate between these two conditions. A recent study demonstrated that, in women with SLE and/or antiphospholipid syndrome (APS), an sFlt1 level <1,872

pg/mL and PlGF >70.3 pg/mL has a negative predictive value of 95% for preeclampsia and for adverse pregnancy outcomes in women with SLE and/or APS with a negative predictive value 95% (226). Unfortunately, delivery may be necessary if immunosuppressive therapy and supportive care fails to stabilize the condition.

The approach to treatment of lupus nephritis during pregnancy is based largely on anecdotal experience and knowledge regarding treatment of lupus in nonpregnant patients.

Glomerulopathies

Glomerulonephritis in Pregnancy

Glomerulopathies may be present in women of childbearing age. They are sometimes not diagnosed before pregnancy but are then detected during routine prenatal care. Glomerulopathies most often encountered in younger women include immunoglobulin A (IgA) nephropathy, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, minimal change disease, and lupus nephritis.

Women in whom proteinuria or hematuria is first detected during pregnancy are likely to have glomerulonephritis, especially in early pregnancy when preeclampsia is rare. However, in the late second and third trimesters, urinary abnormalities and hypertension may also be caused by superimposed preeclampsia. If the serum creatinine is elevated, it is helpful to know the baseline level from either early pregnancy or prepregnancy. Significant elevations in serum creatinine (e.g., >1.5 mg/dL) are unusual in women with preeclampsia, particularly if BP is normal and proteinuria minimal, and are more likely caused by the underlying primary kidney disease. Although most women with preeclampsia usually have only mild reductions in GFR (not greater than 30%), preeclampsia remains the most common cause of acute kidney injury (AKI) in pregnancy (see below).

If glomerulopathy is suspected, evaluation with renal ultrasonography, urinalysis, quantification of proteinuria, and serologic testing is recommended. Renal biopsy can be done, especially early in pregnancy when the patient is more easily positioned on her abdomen. We usually reserve kidney biopsy for women with worsening renal function, and/or heavy proteinuria in whom treatment decisions are likely to be altered. Several case series suggest that kidney biopsy in pregnancy can be helpful in improving the outcome of pregnancy in women with new onset or

worsening CKD in pregnancy (230–232). Bleeding may be more likely because of the gestational increase in renal blood flow, and caution should be exercised when BP is uncontrolled (233–235). In women with near-normal GFR and without debilitating nephrotic syndrome or hypertension, it is usually safe to defer renal biopsy until after delivery. The timing for postpartum biopsy depends on the extent of renal function impairment. Proteinuria may improve once gestational hyperfiltration resolves.

Pregnancy outcomes and prognosis for maternal kidney function are dependent on the baseline GFR, presence of hypertension, and degree of proteinuria (230,236–238). There are few data to suggest an association with histologic diagnosis of glomerulonephritis and differential outcomes in pregnancy (230,231,239). Regardless of the type of glomerulonephritis, it is not uncommon for proteinuria to increase during pregnancy owing to the hemodynamic and hormonal alterations. However, if renal function is well preserved, pregnancy does not appear to adversely affect long-term renal prognosis (at 5 years) (230,237,238). Whether newly diagnosed nephrotic syndrome or worsening proteinuria during pregnancy should be treated has not been prospectively studied, and principles of treatment in nonpregnant women are generally applied. For example, a new diagnosis of minimal change glomerulonephritis in a pregnant woman would be treated with prednisone, whereas IgA nephropathy would most likely be managed conservatively. The optimum strategy for treatment of focal segmental glomerulosclerosis is less clear—oral steroids may be considered if levels of proteinuria are greater than 5 g/day, and if renal function is deteriorating; however, there are few data that specifically address this clinical challenge.

Diabetic Nephropathy

Diabetic nephropathy is a risk factor for poor maternal and fetal outcomes and is present in approximately 5% of pregnant women with type 1 diabetes mellitus (240), particularly those with type 1 diabetes for over 10 to 15 years. Proteinuria may increase during pregnancy, and in women with more advanced CKD, decreases in GFR may occur as pregnancy progresses (241). Women with normal GFR and microalbuminuria at baseline generally have excellent outcomes; however, the risk of preeclampsia and preterm delivery increases with macroalbuminuria and reduced GFR (196,240–243). Women with type 1 diabetes with microalbuminuria, normal renal function, and normotension should be encouraged *not* to postpone pregnancy because of the worse prognosis

once overt nephropathy develops.

Management of pregnant women with diabetic nephropathy includes glycemic control, treatment of hypertension, and close monitoring of maternal and fetal well-being. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are contraindicated during all three trimesters of pregnancy and are associated with a neonatal mortality rate of 25% in the second and third trimesters; therefore, women should be switched to other agents prior to conception (244). Although the evidence is controversial that these agents are associated with fetal malformations with first trimester exposure, we recommend avoiding their use during pregnancy. There is some evidence that optimum control of BP to normal levels is associated with improved pregnancy outcomes in women with diabetic pregnancy (245). Low-dose aspirin reduces the incidence of preeclampsia in women at high risk, and can be considered in women with diabetic nephropathy (243,245,246).

CKD is not as common in women in their childbearing years with type 2 diabetes. A multicenter analysis from Japan suggests that risk factors for adverse maternal and fetal outcomes are similar in women with type 1 and type 2 diabetes. This study demonstrated no differences in the rates of perinatal mortality and congenital malformation between pregnant women with type 1 and type 2 diabetes; however, women with type 2 diabetes displayed a higher risk of primary cesarean section (247). A meta-analysis of pregnancy outcomes in over 500,000 pregnancies reported better outcomes in women with diabetic CKD compared to other etiologies, although the reasons for this are unclear (196).

Urinary Tract-Related Renal Disease

Recurrent UTIs leading to CKD are often caused by anatomic abnormalities of the urinary tract. The most commonly encountered urinary tract-related renal diseases are vesicoureteral reflux (VUR) and chronic pyelonephritis.

VUR nephropathy is a common cause of CKD in young women and is associated with a greater risk for UTI and pyelonephritis during pregnancy. If baseline renal function is abnormal and hypertension present, there is an expected increased risk for preeclampsia; one prospective study of 54 pregnancies reported a 24% incidence of preeclampsia and an 18% incidence of worsening renal function (248,249). Preterm birth risk has been reported to be as high as 30% (249). Renal scarring is thought to be associated with adverse maternal and fetal

outcomes (250–253). The children of women with VUR are at increased risk for VUR, because it is believed to be an autosomal dominant disorder with incomplete penetrance (249).

Given the increased risk of UTI, including pyelonephritis, close monitoring for early detection of bacteriuria with frequent urine cultures (at least once a month or if symptomatic) is recommended. Antibiotics should be initiated if bacteriuria is documented and should be tailored to culture and sensitivity results and should take into account pregnancy or fetal safety profiles.

STONE DISEASE AND PREGNANCY

Symptomatic renal stones are not uncommon in pregnancy; they complicate 1/200 to 1/1,500 of gestations (254–257). Women typically present in the third or second trimester with flank or abdominal pain and hematuria (254–256,258). Renal colic often occurs during pregnancy in women without a prior history of nephrolithiasis, and the diagnosis of renal stone can often be missed (254,259,260).

Gestational elevation in GFR, increased urine supersaturation, hypercalciuria, and decreased ureteral peristalsis should theoretically increase the risk of stone formation (258,261–264). However, the incidence of renal stones in pregnancy is comparable to that in nonpregnant populations. This is possibly caused by increased urinary magnesium, citrate, pH, and nephrocalcin, which protects from stone formation (258,261). The gestational increase in urinary pH and citrate is protective for uric acid and calcium oxalate stones; however, in pregnancy, unlike other populations, the majority of kidney stones are composed of calcium phosphate (265,266).

Renal ultrasonography is the first-line imaging modality for the diagnosis of nephrolithiasis in pregnancy, followed by magnetic resonance urography (without gadolinium) and noncontrast low computed tomography (257). Women with renal colic and hydronephrosis but without visualization of an obstructing calculus on ultrasound may still have a stone on ureteroscopy (20.5%) (267).

Supportive management with analgesics (acetaminophen or opiates but not nonsteroidal antiinflammatory drugs) and hydration is the mainstay of management. Renal calculi will pass or spontaneously fragment in 70% to 84% of cases (259,264,267,268). Ureteroscopy with stenting or stone retrieval is not a very risky procedure but should be performed only when the patient has a large ($^{>}$1 cm) stone, persistent pain, sepsis, worsening

hydronephrosis, an obstructed solitary kidney, or a high-grade obstruction (259,264).

Renal stones in pregnancy have been associated with pyelonephritis, preterm labor, spontaneous abortions, hypertension, and gestational diabetes (255,256,267,269). Interestingly, perinatal outcomes are not significantly altered in women with nephrolithiasis (266,270).

PREGNANCY IN WOMEN WITH END-STAGE RENAL DISEASE

Case reports and case series over the years have clearly documented poor pregnancy outcomes in women with end-stage renal disease (ESRD), and until recently, such pregnancies were rare and hazardous with less than 50% leading to a live birth. Fertility is impaired in women with ESRD at least in part owing to impaired hypothalamic luteinizing hormone release and anovulatory cycles (271). Additional reported abnormalities in reproductive hormones include elevated prolactin levels and decreased levels of estrogen and progesterone (272). Nevertheless, pregnancies do occur, and the pregnancy rate in women with ESRD appears to be increasing, possibly owing to implementation of more aggressive dialysis prescriptions and better control of comorbidities such as anemia and hypertension. In women with advanced CKD, who may not be ready to start dialysis, if pregnancy occurs, many nephrologists will initiate dialysis earlier because of emerging data that more aggressive treatment of the uremic environment is associated with improved maternal and fetal outcomes (273,274).

Hemodialysis

The literature on pregnancy outcomes consists mostly of case reports and case series that have documented a high rate of complications including a very high likelihood for preterm birth (67%–100% incidence), maternal hypertension (20%–66% incidence), IUGR (14%–80% incidence), need for neonatal intensive care (33%–100% incidence), and fetal demise (275). More recently, outcomes have improved most likely in response to increasing the time on dialysis and more aggressive treatment of uremia. A majority of pregnancies treated aggressively now result in live births (81.33%) without major complications (in 75% of pregnancies) (275,276). Some reports suggest that a predialysis BUN of 50 mg/dL or less is associated with a higher rate of live birth and longer gestation (277–279).

Neonates born from women with very elevated BUN levels should be monitored for osmotic diuresis.

A higher dose of hemodialysis (HD) is associated with higher birth weight (211,280,281), gestational age at delivery (211,281), and lower fetal demise rates (281). A comparison of 22 pregnancies from the Toronto Pregnancy and Kidney Disease Registry and 70 pregnancies from the American Registry for Pregnancy in Dialysis Patients revealed that as more HD is administered, the rates of live birth increase significantly (48% live birth rates for women on $\leq$20 hours of HD weekly vs. 85% live birth rate for women on $\geq$37 hours weekly). Nightly HD for 6 to 8 hours was shown to be associated with excellent outcomes in a case series of five patients (282).

There is still no widely accepted dialysis schedule for pregnancy (211,275). Evidence suggests that more dialysis may reduce neonatal death and stillbirth (275). Frequent HD ($\geq$5 times/week) with a total dose of at least 37 hours/week and maintaining BUN $\leq$50 mg/dL is a reasonable and safe strategy, although this schedule is rigorous and may present logistic and financial challenges. Home dialysis technologies (283) may decrease the burden of frequent and prolonged dialysis on pregnant women.

There are no data about the optimal dialysate and heparinization protocol for HD during pregnancy. Minimization of heparin is reasonable in order to prevent obstetric bleeding. In addition, the bicarbonate concentration in the dialysate can be decreased to 25 mEq/L in order to maintain the physiologic metabolic acidosis of pregnancy.

During pregnancy, anemia is often more pronounced, resulting in an increased requirement for erythropoietin and iron supplementation (282,284). There are limited data regarding appropriate management of calcium, phosphorus, and vitamin D in pregnant dialysis patients. Considerations that may provide guidance are that calcium needs increase in pregnancy, increased dialysis may permit liberalization of phosphorus intake, and 1,25-D levels may be increased because of placental 25-hydroxylation of vitamin D. Dry weight titration in pregnancy is challenging because of physiologic volume expansion and edema and should be attempted cautiously.

Peritoneal Dialysis

Peritoneal dialysis (PD) has outcomes that are similar to HD in the general population. However, during pregnancy the equivalence of both treatments

has not been compared rigorously. A meta-analysis of 72 pregnant women on HD and 14 women on PD reported a lower incidence of SGA birth with HD (31%) rather than PD (67%, $P = 0.015$) (211). More data from PD patients are required to verify these findings.

In women who are dialyzed with PD during pregnancy, the exchange volume will need to be decreased and therefore, clearance will need to be achieved by increasing the frequency of exchanges, preferably using a cycler (285). As in nonpregnant women on PD, protein intake should be encouraged and the usual intake recommendation of 1 g/kg/d protein may need to be increased by 20 g/day (282).

Renal Transplantation

Kidney transplantation restores fertility and offers the possibility of near-normal reproductive function including successful pregnancy. Thousands of pregnancies in renal transplant recipients have been reported in case reports and registries since the first successful pregnancy was reported in 1963 (286).

Although pregnancy outcomes are excellent in transplant recipients with near-normal GFR and normal BP, similar to women with CKD, the rate of complications such as preeclampsia and preterm birth is increased. A consensus conference generated a report in 2005 that summarized the literature, proposed practice guidelines, and identified gaps in knowledge (287). Most pregnancies (greater than 90%) that proceed beyond the first trimester succeed; however, there are maternal and fetal complications resulting from immunosuppressant effects, preexisting hypertension, and renal dysfunction. These include maternal complications of steroid therapy such as impaired glucose tolerance, hypertension (47%–73%), preeclampsia (30%), and increased infection. Fetal complications include a 45% to 60% incidence of preterm delivery (mean gestational age is 36 weeks) and IUGR with lower birth weight (average 2.3–2.6 kg). It is recommended that women wait a minimum of 1 year posttransplant to conceive (288). This permits stabilization of immunosuppression regimens, and identification of women with reduced renal function and uncontrolled hypertension in whom pregnancy should be discouraged. Characteristics that are associated with improved pregnancy outcomes include absence of recent rejection, normal BP, absence of proteinuria, preserved renal function (serum creatinine of less than 1.5 mg/dL), being on lower doses of immunosuppressants ($^{\leq}$15 mg/day prednisone, $^{\leq}$2 mg/kg/d, $^{\leq}$5 mg/kg/d cyclosporine), and absence of pelvicalyceal dilatation

(287,289,290).

Pregnancy in a renal transplant recipient should be monitored by a multidisciplinary team of obstetricians, transplant specialists, and internists familiar with the care of pregnant women (291). Monitoring should start before conception, when possible, in order to optimize risk factors and promote normal body mass index, control BP and institute pregnancy-safe immunosuppressive regimens. Similar to women with CKD of any etiology, maternal and fetal outcomes are best when GFR and BP are normal (96,292). The risk of transplant rejection is not significantly elevated, and rejections are often associated with medication nonadherence (292).

Medication-related complications may be encountered during pregnancy. Women on steroid-containing regimens are at risk for impaired glucose tolerance, hypertension (47%–73%), preeclampsia (30%), and infections. Pregnancy itself may lead to the development of antibodies directed at spouse or offspring. This phenomenon may be relevant in women who receive a subsequent transplant from the spouse or offspring, because donor-specific antibody levels may rise after transplantation from such donors, and women receiving human leukocyte antigen antibody-incompatible transplants from their partners or children have a higher rejection rate (293).

The impact of immunosuppression on maternal and fetal outcomes has been assessed with respect to fetal malformations, preterm birth, and hypertensive complications. A recent report suggested that the risk of preeclampsia was also increased in pregnancies fathered by males on immunosuppression after kidney transplantation (294).

Calcineurin inhibitors are used widely and considered safe, but are associated with a higher rate of preeclampsia and gestational hypertension compared to azathioprine, and possibly with slightly shorter gestations. Tacrolimus levels (both free and albumin bound) are altered during pregnancy as a result of multiple factors including anemia and hypoalbuminemia (295), thus making interpretation of whole blood levels difficult (296). It is likely that in many women, tacrolimus availability is increased despite lower whole blood trough levels. Suggested strategies for dosing include not adjusting the dose until whole blood levels reach 50% of prepregnancy levels, or maintaining the whole blood level constant and carefully monitoring for signs of drug toxicity. Sirolimus is classified as a pregnancy risk C medication, and its safety is not established in human pregnancies (297). Mycophenolate mofetil is embryotoxic in animals and has been associated with ear deformities, cleft lip/palate, and first trimester

spontaneous abortions in human pregnancies (298), and should be avoided in pregnancy and in women who are planning to become pregnant at least 6 weeks before conception. Azathioprine can be used instead of mycophenolate mofetil in these women.

Detailed immunologic studies have not been reported in either pregnant renal transplant recipients or their offspring. Acute graft rejection during pregnancy occurs uncommonly and may be difficult to diagnose and differentiate from other causes of AKI or preeclampsia. Transplant biopsy may be necessary to make an accurate diagnosis; however, the logistics are complicated in late pregnancy by the enlarging uterus and if renal function is deteriorating, early delivery may be necessary. The treatment for rejection may include intravenous steroids and intravenous immunoglobulin (287). The safety of antilymphocyte antibodies and rituximab is not well established in pregnancy (287).

Transplant Donor

In recent years, several studies have raised concerns that women who donate a kidney may be at an increased risk of developing preeclampsia in later pregnancies (299–301). The incidence of preeclampsia was 11% in donors compared to 5% in a matched control group. Despite the increased incidence of preeclampsia and gestational hypertension in donors, there was no statistically significant difference in pregnancy outcomes: preterm birth and birth weight (301). Overall, it is important to discuss the increased risk of preeclampsia with any female kidney donor of reproductive age.

Pregnancy-Specific Renal Disorders

HYPERTENSION IN PREGNANCY

Hypertension is the most common complication of pregnancy and affects 6% to 10% of pregnancies (302). Hypertension is potentially a highly morbid and even lethal complication of pregnancy, and although rare, most pregnancy-related strokes are attributable to hypertension (303,304).

Hypertension in pregnancy is defined as a BP of 140/90 mm Hg or above. Mild to moderate hypertension refers to BP 140 to 159/90 to 109, and severe hypertension refers to BP 160/110 mm Hg or above (305).

Although several different classification schemas are used worldwide,

the American College of Obstetricians and Gynecologists (ACOG) and the National Hypertension Education Program endorse the distinction of four major categories (101): preeclampsia-eclampsia; chronic hypertension; chronic hypertension with superimposed preeclampsia; and gestational hypertension.

Preeclampsia

Clinical Manifestations and Diagnosis

Preeclampsia affects 2% to 5% of pregnancies (306–308) and is responsible for 12% to 15% of maternal deaths (309). The traditional hallmarks of preeclampsia include new onset hypertension in the latter half of pregnancy with proteinuria. More recently, the ACOG has acknowledged that nonproteinuric forms of preeclampsia occur and have modified their diagnostic criteria to that effect (101). Thus, their recommended diagnostic criteria include new onset hypertension (BP $\geq$140/90 mm Hg) after 20 weeks of gestation and one of the following symptoms/signs, which cannot be attributed to another disease process:

- De novo proteinuria: $>$0.3 g/day on 24 hours urine collection or urine protein/creatinine ratio $>$0.3. Both quantification methods are considered adequate (101,310–312). A dipstick with 1^+ protein can be used alternatively only when quantitative testing is not available.
- Neurologic symptoms
- Thrombocytopenia: platelets $<$100,000/*m*L
- Pulmonary edema
- Transaminitis: alanine aminotransferase (ALT) or aspartate aminotransferase (AST) above twice the normal range
- Renal insufficiency: creatinine $>$1.1 mg/dL or doubling from baseline.

Severe features of preeclampsia include: BP $\geq$160/110 mm Hg, platelet count $<$100,000 µL, pulmonary edema, transaminitis, severe right upper quadrant pain, creatinine $>$1.1 mg/dL or doubling, or new onset of cerebral or visual symptoms. The HELLP (Hemolysis, Elevated Liver enzymes, Low Platelets) syndrome and eclampsia (features of preeclampsia and seizures) are both considered to be severe variants of preeclampsia rather than separate diseases.

Risk Factors for Preeclampsia

Risk factors for preeclampsia include (313–316):

- African American race
- chronic hypertension (especially hypertension secondary to renovascular hypertension, pheochromocytoma or primary aldosteronism)
- thrombophilia
- diabetes mellitus
- chronic kidney disease
- obesity
- prior history of preeclampsia
- family history of preeclampsia
- primigravida
- maternal age $^{>}$40 years
- multifetal pregnancy

Pathophysiology of Preeclampsia

The last decade has seen progress in our understanding of the pathophysiology of preeclampsia, although there remain significant gaps. Preeclampsia may be conceptualized as a two-stage process; the placenta is central to the pathophysiology of the syndrome, and this early stage is characterized by impaired uteroplacental blood flow (317). Studies suggest that defective remodeling of the terminal branches of the uterine artery (spiral arteries) is a fundamental lesion that then results in impaired placental perfusion. During normal pregnancy, placental cytotrophoblasts relocate from chorionic villi and invade the uterus, reaching the inner third of the myometrium and deeply invade the spiral arteries. Cytotrophoblasts migrate up these vessels and replace the maternal endothelial lining (317). The spiral arteries then acquire physiologic properties that permit adequate placental perfusion. In preeclampsia, cytotrophoblast invasion of the interstitial uterine compartment is shallow, although this is a variable finding. In many locations, spiral artery invasion is incomplete and some vessels retain portions of their endothelial lining with relatively intact muscular coats, although others are not modified. This impaired placental perfusion is believed to initiate a sequence of events that include (317) immune dysregulation, ischemia, and release of angiogenic factors into the maternal circulation, setting the stage for the second stage of preeclampsia,

which encompasses the multifaceted maternal syndrome (318). The hallmark of the maternal syndrome is vascular endothelial injury.

Several circulating placental derived factors may contribute to maternal vascular endothelial injury, including sFlt-1, soluble endoglin (319), hypoxia-inducible factor-1 (HIF-1), syncytiotrophoblast debris, and various cytokines. Angiotensin II type 1 (AT1) receptor agonistic antibodies have also been reported to be involved in the pathogenesis of preeclampsia in animal models as well as in clinical cases of preeclampsia (320). Elevations of sFlt-1 and endoglin have been reported prior to and at the time of clinical disease (321,322). sFlt-1 is a circulating antagonist of VEGF released from the placenta, and is hypothesized to contribute to maternal vascular endothelial dysfunction and to cause glomerular endotheliosis (323). PlGF, an angiogenic factor similar to VEGF, is decreased in women with preeclampsia. The endothelial damage caused by placentally derived circulating factors results in maternal hypertension and dysfunction in the kidney, liver, and brain.

Preeclamptic Hypertension

Elevated BP is often the first clinical sign that preeclampsia is present; it is the clinical feature that is frequently the proximate cause of preterm delivery, and also an important risk factor for maternal intracerebral hemorrhage (324). Subtle increases in BP are detectable weeks before preeclampsia is diagnosed in women who develop the disease (325). In many women, it is the defining feature of the syndrome, with severe BP elevations that persist for days or weeks postpartum, whereas in others the hypertension is mild, asymptomatic, and disappears quickly after delivery. As mentioned above, normal pregnancy is characterized by early, marked vasodilation and lower BP compared to the case when nonpregnant. When preeclampsia develops, there is early and sustained suppression of the circulating renin–angiotensin system (36,47,326), and there are lower levels of relaxin (327) and altered levels of estrogen and progesterone (328), suggesting that decreased vasodilation contributes to preeclamptic hypertension. There is also evidence for excessive vasoconstriction with increased endothelin (329), deficient prostacyclin (330), increased sympathetic nervous system activity (59–61), and, as mentioned, agonistic autoantibodies to the AT1 receptor in some cases (331). Preeclampsia shares some clinical features with acute glomerulonephritis such as acute onset of hypertension, mildly reduced GFR, proteinuria, and edema. Although hypertension associated with glomerulonephritis is

heterogeneous, clearly in some patients there is a sodium volume component in patients with acute decrements in GFR; the suppressed PRA (36) and increased ANF (332), which are features of preeclampsia, are consistent with excess volume (333,334). Underlying genetic heterogeneity and predisposition may play a role in the degree to which a woman will manifest hypertension when she develops preeclampsia, and this may explain some of the earlier genetic association studies that reported associations between single nucleotide polymorphisms in the angiotensinogen gene (335), angiotensin-converting enzyme gene (336), genes involved in the nitric oxide pathway (337), and risk for preeclampsia.

Serum levels of sFlt-1 are increased in women several weeks prior to the development of preeclampsia and until delivery, after which they fall rather quickly (338,339). sFlt-1 antagonizes the proangiogenic biologic activity of circulating VEGF and PlGF by binding to them and preventing their interaction with their endogenous receptors (323). Along with the increased circulating sFlt-1 levels, both PlGF and VEGF levels are decreased in women with preeclampsia. As VEGF is necessary for healthy vascular endothelial function, a plausible link between placental dysfunction and hypertension in preeclampsia is the presence of antiangiogenic factors, which, by blocking VEGF, cause endothelial dysfunction and hypertension. Support for this hypothesis includes the numerous clinical studies, demonstrating increased levels of sFlT-1, decreased PlGF and VEGF in women with preeclampsia, and a rising sFlt-1/PlGF ratio preceding the clinical manifestations of the syndrome (323,340–344). Also, experimental models of preeclampsia demonstrate that preeclampsia can be reproduced in a rodent model with administration of exogenous sFlt-1 (339).

Another particularly intriguing observation is that patients treated with chemotherapeutic agents that target the VEGF signaling pathway develop a preeclampsia-like syndrome characterized by hypertension, proteinuria, central nervous system dysfunction resembling the posterior reversible leukoencephalopathy syndrome and, at times, microangiopathy (345,346). The mechanism of antiangiogenic therapy-induced hypertension demonstrates similarities to that observed in women with preeclampsia—decreased nitric oxide production and increases in circulating endothelin levels (346). In experimental models, administration of an endothelin receptor antagonist has been shown to prevent the development of antiangiogenic therapy-induced hypertension (347). Angiogenic factors, especially sFlt-1 and PlGF, are promising biomarkers for preeclampsia but

remain a research tool as they are not commercially available in the United States. Their use has shown promise in distinguishing worsening hypertension, lupus, or CKD from preeclampsia (212).

Renal Manifestations of Preeclampsia

Glomerular endotheliosis (Fig. 13-3) is a pathognomonic, yet not exclusive, feature of preeclampsia (348–350). Experimental as well as clinical data suggest that increased circulating levels of sFlt-1 and soluble endoglin may contribute to the pathogenesis of glomerular endotheliosis by binding to circulating VEGF. Glomerular endotheliosis is similar to the lesion described in patients who have developed proteinuria after treatment with antiangiogenic chemotherapy (7,323,351) and is characterized by a reduced number of fenestrae (352) and anionic binding sites (353,354).

Renal biopsy studies performed within 48 hours of delivery on preeclamptic women, with a slightly decreased GFR (mean GFR of 91 $\pm$ 25 mL/min/1.73 m^2 compared to 149 $\pm$ 34 mL/min/1.73 m^2 in control healthy pregnant women) demonstrate thickened basement membrane, reduced number of fenestrae and anionic binding sites, as well as subendothelial fibrinoid deposits that lower glomerular permeability (352,353). In addition, mesangial cell interposition restricts the filtration area and lowers the ultrafiltration coefficient (K_f), leading to the conclusion that the reduced GFR in preeclampsia has a primarily structural basis with a contribution of reduction in RPF (23,78,352).

Serum creatinine rarely rises to levels that are considered abnormal for nonpregnant individuals in women with preeclampsia, and in most women, serum creatinine is $<$1.2 mg/dL—a level that, although not dramatic, would represent a 50% decrement from normal pregnancy levels, which are, on average, about 0.60 mg/dL.

Preeclamptic Proteinuria

Considered for decades to be one of the key diagnostic features of preeclampsia, proteinuria is present in most women who are diagnosed clinically as having preeclampsia. Although it is well recognized that women with hypertension in the second half of pregnancy and evidence of other systemic manifestations of preeclampsia such as the HELLP syndrome may not always manifest proteinuria, from the nephrologist's perspective it remains an important feature of the disorder. As in most glomerular diseases, most of the protein excreted in the urine of

preeclamptic women is albumin. The precise mechanisms for preeclamptic proteinuria had, until recently, been somewhat of a mystery. Until recently, the descriptions of the pathophysiology of preeclamptic proteinuria have emphasized alterations in the glomerular basement membrane and the endothelial cells. As already mentioned, VEGF plays an important role in vascular endothelial integrity. The elevated sFlt-1 levels in preeclampsia, which lead to reduced VEGF, are a likely candidate mechanism for preeclamptic proteinuria, a hypothesis supported by the animal studies demonstrating that reductions in VEGF lead to a renal lesion with histologic characteristics of glomerular endotheliosis (355). Moreover, recent small studies of extracorporeal removal of sFlt-1 in women with early preeclampsia demonstrated that protein to creatinine ratios rose and fell in conjunction with sFlt-1 levels (356,357). Again, the analogy to cancer patients treated with anti-VEGF therapy is informative; proteinuria induced by these medications often resolves after cessation of the drug.

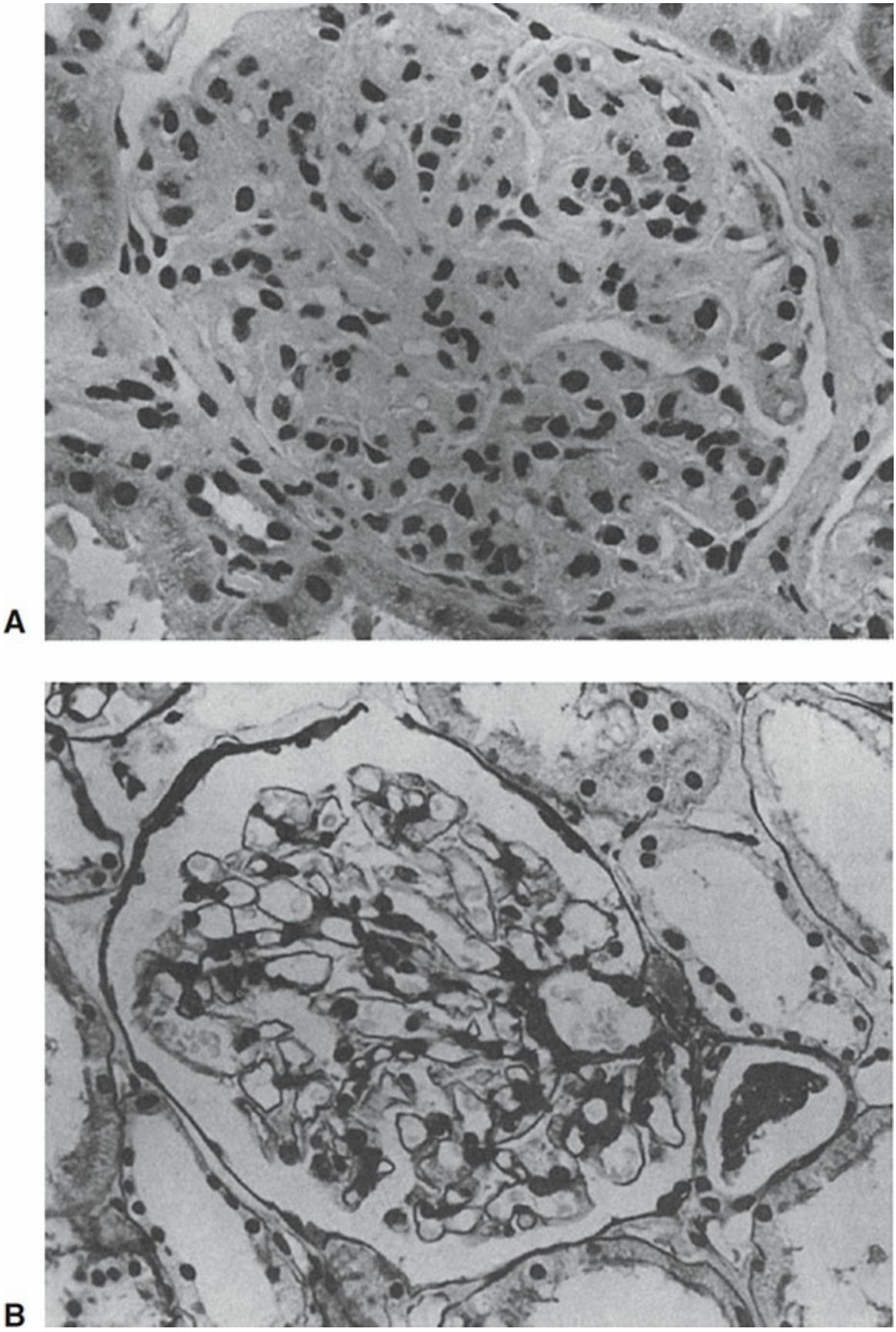

Figure 13–3 Glomerular endotheliosis and normal glomerulus seen on light microscopy. (**A**) Glomerular endotheliosis. (**B**) A normal glomerulus. (From

Venkatachatam MA, Kriz W. Anatomy. In: Heptinstall RH, ed. *Pathology of the Kidney*. 4th ed. Wolters Kluwer ¿ 1992.)

Further studies have gone on to expand our knowledge of preeclamptic proteinuria. Garovic and colleagues reported increased excretion of podocytes in the urine of women with preeclampsia (358). They also reported decreased glomerular expression of nephrin and synaptopodin in kidney tissue from autopsies of women with preeclampsia (359). These observations have been confirmed, and others have also found increased serum and urinary nephrin levels, and increased urinary excretion of podocalyxin in preeclamptic women compared to normal pregnant women (360,361). Thus, a plausible scenario emerges whereby elevated levels of sFlt-1, released by an underperfused placenta, lead to VEGF depletion, which then contributes to alterations in the glomerular filtration barrier, including disruption of glomerular endothelial cells and podocyte injury/depletion. Podocyte shedding in the urine may be the marker for the latter phase of injury.

Additional renal manifestations of preeclampsia include elevated serum uric acid $^{>}$5.5 mg/dL (327 *m*mol/L) presumably because of decreased renal clearance. Indeed, the fractional clearance of uric acid decreases, often preceding the appearance of overt disease (362). Marked hypocalciuria has been consistently observed and is likely caused by mildly reduced GFR and enhanced tubular reabsorption. Serum-ionized calcium is normal or slightly decreased. PTH concentration is elevated, although reports are conflicting. Serum 1,25-D is decreased probably secondary to reduced placental and/or renal production. This leads to reduced intestinal calcium reabsorption and a state of calcium deficiency, thereby increasing PTH and distal tubular reabsorption of calcium.

A number of cases of hyponatremia have been reported in women with preeclampsia (363–371). The mechanism of decreased free water excretion in preeclampsia has not been clarified; however, reports of increased copeptin levels may be relevant (138,372,373).

Multisystem Pathophysiologic Alterations in Preeclampsia

Preeclampsia leads to multiorgan involvement with variable presentations. We will briefly describe the main extrarenal pathophysiologic features that have been associated with this syndrome.

Women with preeclampsia have an increase in CO early in pregnancy that is followed by a decreased CO in the third trimester (374–380).

Peripheral vascular resistance (381) and central venous pressure (382) are increased in preeclampsia. This is associated with an increased cardiac afterload and impaired diastolic function (383,384). Peripartum heart failure can occur in this setting, although it is usually a complication of preexisting heart disease.

Eclamptic seizures can occur with modest elevations in BP. Indeed, preeclampsia is associated with an impaired cerebral blood flow autoregulation (385), which persists even after lowering systolic BP below $^{<}$140 mm Hg with medication (386). Severely elevated BP is a risk factor for intracerebral hemorrhage in women with preeclampsia (324). Posterior reversible encephalopathy syndrome is often found on magnetic resonance imaging in eclampsia (387,388), especially in younger women with thrombocytopenia or proteinuria (388). Headaches precede 64% of seizures, whereas visual changes are present in only 32% of cases (389). Therefore, both symptoms should prompt rapid evaluation during pregnancy and in the postpartum period.

Management of Preeclampsia

A detailed discussion of obstetric and medical management is beyond the scope of this chapter.

Important principles include early diagnosis and close maternal and fetal surveillance with appropriately timed delivery.

Treating severe range hypertension (BP $^{>}$160/110 mm Hg) with antihypertensives has been shown to decrease the risk of intracerebral hemorrhage and maternal death (302). In order to further prevent adverse pregnancy outcomes, we suggest preventing the development of severe hypertension by initiating oral antihypertensive therapy when BP reaches $^{\geq}$150/95 mm Hg.

In preeclamptic women with central nervous system involvement (hypertensive encephalopathy, intracranial hemorrhage, eclampsia), intravenous antihypertensives are indicated to decrease MAP by 25% in minutes to hours and to 160/100 mm Hg or less over subsequent hours (302). The target for BP control in severe hypertension is to maintain BP in the 140 to 150/90 to 100 mm Hg range (305).

The ACOG has identified three first-line agents for the treatment of severe hypertension: intravenous labetalol, intravenous hydralazine, and oral nifedipine (305). The second-line therapy for the treatment of severe hypertension that fails to respond to first-line agents includes the consultation of a specialist and a continuous infusion of labetalol or

nicardipine (305).

The decision to deliver is often very difficult to make when the fetus is preterm, especially before 34 weeks of gestation. In the early preterm period (23–34 weeks), the goal is to prolong pregnancy as much as possible with BP control and bed rest. Delaying delivery to a higher gestational age will improve fetal outcomes. Nevertheless, signs of serious maternal disease (headache, abdominal pain, or HELLP syndrome) or fetal distress are indications for delivery, and delays may lead to poor pregnancy outcomes (390). Epigastric and chest pain should trigger a thorough workup in women with preeclampsia because hepatic rupture and hepatocellular necrosis can occur in HELLP/preeclampsia. Women with HELLP syndrome are at a high risk for poor pregnancy outcomes and should be delivered emergently.

At 34 to 37 weeks of gestation, expectant management is also preferred, and delivery is delayed until $\geq$37 weeks (391,392) unless severe features of preeclampsia or fetal indications that would prompt delivery are present. In women who are at a gestational age of 38 weeks or more, delivery for preeclampsia is indicated.

Prevention of Preeclampsia

Aspirin, at a low dose, administered early in the first trimester, is associated with a modestly decreased risk of preeclampsia (˙20%) (393–395) and is recommended in women who are at a high risk for preeclampsia (246,396).

Calcium supplementation of 1 g or more per day may decrease the risk of preeclampsia but only in women with low dietary intake (164).

Sodium restriction, dietary protein, fish oil, oral magnesium all failed to show a reproducible and statistically significant decrease in the risk of preeclampsia in clinical trials.

Intravenous magnesium sulfate is indicated for the prevention of seizures only in women with eclampsia, severe preeclampsia, and preeclampsia requiring C-section (101).

Treatment with LMWH has been investigated for the prevention of preeclampsia in high-risk groups but was not of clear benefit (397–400). However, women with a genetic or acquired thrombophilias (factor V Leiden, prothrombin gene G20210A, lupus anticoagulant) may benefit from LMWH therapy in pregnancy (401,402). Interestingly, a Cochrane review in 2014 failed to see a benefit for LMWH in women with a prior history of recurrent miscarriage whether or not they had an underlying

thrombophilia (403). At this time, the clinical trial evidence is inconclusive, benefits of LMWH are only apparent in certain subgroups or case series, and data from larger randomized clinical trials are needed for confirmation (404).

Chronic Hypertension in Pregnancy

Pathophysiology and Clinical Manifestations of Chronic Hypertension in Pregnancy

Chronic hypertension is defined as hypertension that is diagnosed before pregnancy, or prior to 20 weeks of gestation (101). The estimated prevalence is 3.6% to 9.1% (405). The majority (89%) of women with chronic hypertension, however, have essential hypertension (406); women with chronic hypertension should undergo a workup for potential secondary causes if hypertension is severe, or if there are renal or electrolyte abnormalities, preferably before conception.

Chronic hypertension in pregnancy is associated with higher rates of maternal and perinatal morbidity and mortality. This has been confirmed in a number of recent large, population-based studies (406,407). However, these and other reports have not consistently distinguished between women with preeclampsia superimposed on chronic hypertension and pregnant women with uncomplicated chronic hypertension alone (408–411). Although there is little doubt that women with superimposed preeclampsia have higher rates of adverse maternal and fetal/neonatal outcomes, the independent risks associated with uncomplicated chronic hypertension are less clear. Chronic hypertension is also associated with increased risk of gestational diabetes (odds ratio 1.8, 95% confidence interval 1.4–2.0). This may reflect common risk factors for both conditions such as obesity as well as similar pathogenic mechanisms, e.g., insulin resistance (410).

Placental abruption, which is associated with life-threatening maternal hemorrhage, is estimated to be threefold higher in women with chronic hypertension, although most of this risk is associated with superimposed preeclampsia (408,412,413). Other studies have not demonstrated an increased risk of abruption in women with chronic hypertension without superimposed preeclampsia (411,414,415). Differences in sample size and study population may account for the varying results.

Other adverse maternal outcomes include accelerated hypertension during pregnancy with resultant target organ damage, e.g., kidneys, heart,

and brain, although in the absence of superimposed preeclampsia, this is extremely uncommon. One exception may be women with severe hypertension prior to conception, many of whom have underlying renal disease or secondary hypertension. Some women with secondary forms of hypertension, such as chronic renal disease and collagen disorders, may suffer from irreversible deterioration in renal function during and after pregnancy. In the case of SLE, there may be multiorgan morbidity, regardless of the development of superimposed preeclampsia.

Finally, although the expectation is that pregnancies in women with uncomplicated chronic hypertension will be successful, these women are more likely to be hospitalized for hypertension (414).

Perinatal mortality is also higher in pregnancies complicated by chronic hypertension, with most of this increased risk attributable to superimposed preeclampsia and fetal growth restriction (408,409,414).

Chronic Hypertension with Superimposed Preeclampsia

Superimposed preeclampsia is likely when any of the following are present (101):

- A sudden increase in BP that was previously well controlled or escalation of antihypertensive medications to control BP.
- New onset of proteinuria or sudden increase in proteinuria in a woman with known proteinuria before or early in pregnancy.

As discussed, pregestational hypertension is a recognized risk factor for preeclampsia. The incidence of superimposed preeclampsia ranges from 13% to 40% and is greater in women with more severe hypertension of longer duration, and in women with secondary forms of hypertension (101,408,416,417).

An intriguing question is why women with preexisting hypertension are at greater risk for the development of superimposed preeclampsia. It has been suggested that women at risk for preeclampsia have genetic, biochemical, and metabolic abnormalities similar to women with essential hypertension (418). This list includes a higher incidence of polymorphisms in the angiotensinogen gene, obesity, hypertriglyceridemia, and insulin resistance.

Recent paradigms of the pathogenesis of preeclampsia emphasize that there are necessary fetal as well as maternal susceptibility factors. Elevated BP, considered to be a “maternal susceptibility factor,” clearly increases

risk; however, fetal/placental pathologic abnormalities are necessary for the full expression of the disease. Dysregulation of angiogenic factors is a feature of preeclampsia developing in previously normotensive women (refer to the Pathophysiology of Preeclampsia section). We and others have found that maternal serum levels of angiogenic factors are altered in women with chronic hypertension and superimposed preeclampsia, similar to women with preeclampsia without preexisting hypertension (419–421). An analysis of 313 women with chronic hypertension demonstrated that sFlt-1 and endoglin were significantly elevated between 26 and 30 weeks in women with superimposed preeclampsia compared to women with chronic hypertension who did not develop superimposed preeclampsia (422). Overall, the data suggest similarities in pathogenesis between preeclampsia in previously normotensive women and those with superimposed preeclampsia. Further study is needed to evaluate the contribution of other etiologies in this subgroup of women.

Therapeutic Goals

A comprehensive review of management is beyond the scope of this chapter. The reader is referred to the recent ACOG task force and other scholarly reviews (101,302,423–427). We highlight some of the principles of treatment of hypertension in pregnancy.

BP lowering using antihypertensive medications has been successful at decreasing the incidence of severe hypertension (425). In women with preeclampsia, severe range hypertension (BP $\geq$160/110 mm Hg) significantly increases the risk of cerebrovascular events (324). There is no clear evidence that treating mild to moderate hypertension improves outcomes in terms of superimposed preeclampsia (425,428), preterm birth (429), or SGA (425,430).

The BP level at which pharmacologic therapy should be initiated in pregnancy is debated. Current guidelines vary and recommend the initiation of antihypertensive drugs at levels ranging from BP $\geq$140 to 160/90 to 110 mm Hg (424,426). Recommendations from major American and European societies are summarized in Table 13-1. We advise the initiation of antihypertensive medications when BP $\geq$150/90 to 100 mm Hg (302), regardless of the type of hypertension (431). Lower thresholds may be appropriate in women with symptoms, or in those with target organ damage.

There is also controversy regarding the appropriate treatment target. The ACOG recommends a BP target range of 120 to 160/80 to 105 mm Hg

(101). The Canadian guidelines individualize treatment in women with chronic hypertension in pregnancy according to the presence or absence of comorbidities and identify a BP goal of <140/90 mm Hg and 130 to 155/80 to 105 mm Hg, respectively (426).

The Control of Hypertension in Pregnancy Study (CHIPS) compared a lower (130/80) to a higher (140/90) in 987 pregnant women with nonproteinuric hypertension (75% chronic hypertension, 25% gestational hypertension) and demonstrated that the lower BP target was safe (432). This finding discredited previous concerns for fetal harm if BP were to be reduced to the normal range (433). Furthermore, there were fewer episodes of severe hypertension in women treated to the lower BP target (432). Table 13-2 summarizes the characteristics of the most commonly used antihypertensives in pregnancy.

Acute Kidney Injury in Pregnancy

In the United States, AKI from obstetric causes is an unusual event and affects less than 1 in 20,000 pregnancies (434). Obstetric causes for AKI in the first trimester (between 12 and 18 weeks) include septic abortion and hyperemesis gravidarum (which causes prerenal azotemia). In the third trimester and in the puerperium, obstetric causes of AKI include preeclampsia and HELLP syndrome (refer to the Renal Manifestations of Preeclampsia section), acute fatty liver of pregnancy (AFLP), bleeding complications (leading to prerenal azotemia, acute tubular necrosis, or renal cortical necrosis), obstruction and, very importantly, nonsteroidal antiinflammatory use for postpartum pain control.

Table 13–1 Blood Pressure Thresholds to Initiate Antihypertensive Medication during Pregnancy Complicated by Hypertension

Recommending Entity	Start Treating When Either BP Value is ≥	Consider Therapy if
American College of Obstetricians and Gynecologists' (ACOG) Task	160/105 mm	

Force on Hypertension in Pregnancy (101)		
American Heart Association /American Stroke Association (AHA/ASA) (427)	160/105 mm Hg	
The Society of Obstetricians and Gynaecologists of Canada (426)	160/110 mm Hg	150–159/100–109 mm Hg
Task Force on the Management of Cardiovascular Diseases during Pregnancy of the European Society of Cardiology (ESC) (424)	150/95 mm Hg	140/90 mm Hg in women with: gestational hypertension alone or superimposed to chronic hypertension and organ damage from elevated BP (subclinical or symptomatic)
Royal College of Obstetricians and Gynaecologists (423)	150/95 mm Hg	
National High Blood Pressure Education Program (NHBPEP) Working Group on High Blood Pressure in Pregnancy (302)	150/90–100 mm Hg	

Table 13–2 Antihypertensive Drugs Used for the Management of Chronic Hypertension

Drug (FDA Category)	Mechanism of Action	Dose	Maximum Dose	Comments
Methyldopa (B)	Centrally acting α_2-receptor agonist	500–3,000 mg/day in 2–3 divided doses; generally start with	3 g/day	Preferred agent of National High Blood Pressure Working group; slow onset; side effect profile includes

	agonist	start with 250 mg po twice daily		includes lethargy; bes long-term data for children exposed in utero
Labetalol (C)	α- and β-adrenergic antagonist	100–2,400 mg/day in 2–3 divided doses; generally start at 100–200 mg p.o. twice daily	2,400 mg/day	Increasingly preferred as a first-line agent; rapid onset of action; avoid in patients with asthma or congestive heart failure
Nifedipine (C)	Calcium-channel blocker	30–120 mg/day of a slow release preparation	120 mg/day	Side effects include headache, flushing, tachycardia; once a day dosing may improve compliance
Hydrochlorothiazide (C)	Thiazide diuretic	12.5–50 mg/day	50 mg/day	Not used as a primary agen in pregnancy considered a adjunctive agent; theoretical concerns of reduced intravascular volume and decreased uterine blood flow in pregnancy; electrolytes

				monitored
Hydralazine (C)	Vasodilation, smooth muscle relaxant	50–300 mg/day in 2–4 divided doses	300 mg/day	Not used as a primary agen in pregnancy considered an adjunctive agent; may b used in combination with a sympatholyti agent (e.g., methyldopa or labetalol) to prevent tachycardia

Nonobstetric causes of AKI in pregnancy are similar to the ones present in nonpregnant populations and therefore may be worked up similarly. As previously stated, renal biopsy is not contraindicated during pregnancy (231,232). As for any invasive procedure during pregnancy, the risks and benefits need to be clearly outlined. The risk of pregnancy loss was 5/82 when combining four case series of biopsy in pregnant women, and complication rates were low (231,232,350,435). Renal biopsy may be of great benefit in cases when it affects the mother's decision to continue or terminate her pregnancy, or gives information about maternal/fetal outcomes, and it can often alter the treatment of AKI (231,435).

Indications for HD initiation in pregnancy are discussed in the Hemodialysis section. Women who require HD for AKI during pregnancy will most often recover renal function within 6 months postpartum (436,437).

Volume Depletion

Pregnant women can develop volume depletion similarly to nonpregnant populations. Women with hyperemesis gravidarum have large volume losses and poor oral intake; they are thus at a high risk of developing volume depletion and azotemia.

Uterine hemorrhage is a pregnancy-specific cause of blood loss that can occur near term and is often concealed. It is not uncommon for the

can occur near term and is often concealed. It is not uncommon for the blood loss to be underestimated; therefore, physicians should promptly react to any overt bleeding and transfuse when needed.

Obstruction

During pregnancy, the ureters appear dilated and are partially obstructed (refer to the Renal Anatomy during Normal Pregnancy section). This is often more prominent on the right side but is rarely a cause of significant obstruction or AKI. Obstructive AKI during pregnancy is more often caused by massive oligohydramnios, multiple gestations, large uterine fibroids, or obstructing renal calculi. When a bilateral obstruction is causing a significant renal impairment, ureteric stents may be placed until delivery. Renal stones during pregnancy are discussed in the Stone Disease and Prevention section of this chapter.

Bilateral Renal Cortical Necrosis

Renal cortical necrosis is a rare but morbid cause of obstetric AKI usually seen in the context of hemorrhage caused by placental abruption, placenta previa, or any cause of postpartum hemorrhage, including uterine rupture. It is defined as ischemic destruction of the renal cortex secondary to a prolonged decrease in renal arterial perfusion and follows disseminated intravascular coagulation (DIC) and severe renal ischemia (438). Symptoms include oliguria or anuria, hematuria, and flank pain. Renal cortical necrosis is suspected when AKI is severe and lasts more than 4 weeks and often results in the need to initiate dialysis (436,439). Imaging (ultrasound or computerized tomography or MRI) may confirm the diagnosis in 50% of cases by demonstrating hyperechoic or hypodense areas of necrosis in the renal cortex and calcification of the medullary-cortical junction. It is more often encountered in developing countries, but its incidence and morbidity have significantly decreased over the past decades (439,440). A recent case series from France highlighted the fact that although this entity is rare, outcomes were poor, with 39% of 18 cases progressing to ESRD, and none of the patients recovered normal kidney function (438). The authors of this case series postulated that the development of renal cortical necrosis may have been associated with prolonged exposure to the antifibrinolytic agent, tranexamic acid.

Although rare, thrombotic microangiopathies (thrombotic thrombocytopenic purpura [TTP] and hemolytic uremic syndrome [HUS]) are an important cause of pregnancy-associated AKI because they are associated with considerable morbidity (441). These disorders are usually characterized by elevated BP, hemolytic anemia, thrombocytopenia, and renal insufficiency. On renal histopathology, there is thrombotic microangiopathy (TMA) with platelet or fibrin microthrombi occluding small arterioles or capillaries. TTP is due to a congenital or acquired deficiency in ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), a metalloproteinase enzyme that cleaves von Willebrand factor (442). Although in nonpregnant children and adults, HUS is often due to Shigatoxin-producing bacteria (associated with diarrheal illness), in pregnancy HUS is now recognized to be associated with mutations in genes involved in regulation of the alternate complement activation pathway and is referred to as atypical HUS (aHUS) (442). Uncontrolled complement activation leads to complement-induced endothelial damage that promotes TMA. A number of genetic mutations have been reported, including inactivating mutations in genes coding for the inhibitory factors of complement activation: factor H, factor I, and membrane-cofactor protein, as well as gain-of-function mutations in the genes coding for the two main components of the alternative C3 convertase, C3 and FB, which renders C3 convertase resistant to inhibition (443). Many of these mutations do not have complete penetrance and require a secondary event for the disease to manifest itself. Pregnancy is an important trigger for TMA, and pregnancy-associated TMAs constitute 8% to 18% of all cases of TMA (443–447). Women with a history of TTP have a 30% to 60% risk of a relapse later in life that is often triggered by pregnancy, infection, or surgery (448–450). For women with a known complement gene abnormality, the risk of developing aHUS during pregnancy will vary between 10% and 30% depending on the specific mutation (442,446,444,451). Women with a history of TTP or complement gene abnormality are also at an increased risk of developing preeclampsia or HELLP syndrome (450,452).

TTP and aHUS share several clinical and laboratory features of the HELLP variant of preeclampsia and AFLP (442). Differentiating between these etiologies is important in guiding diagnosis or establishing a prognosis. Features that may be helpful in establishing the correct diagnosis include timing of onset and the pattern of laboratory abnormalities.

diagnosis include timing of onset and the pattern of laboratory abnormalities.

Preeclampsia is far more common than TMAs and typically develops in the third trimester, with only a few cases developing a few days postpartum. TTP generally occurs in the second or third trimester. HUS is usually a postpartum disease (443), which can make it difficult to differentiate from postpartum preeclampsia with severe thrombocytopenia or HELLP syndrome.

Clinically, preeclampsia spontaneously recovers after delivery, whereas TTP/HUS is often but not always associated with persistent thrombocytopenia, renal insufficiency, and hypertension, with many patients requiring dialysis or transplantation long term (453–456). AKI, although not unusual with AFLP, usually does not progress to end-stage kidney failure unless significant bleeding or hemodynamic instability or marked DIC occurs.

In contrast to TTP/HUS, preeclampsia is usually preceded by hypertension and proteinuria; it can also be associated with mild DIC and prolongation of prothrombin and partial thromboplastin times. Another laboratory feature of preeclampsia/HELLP syndrome that is not usually associated with TTP/HUS is marked elevations in liver enzymes. The presence of fever is more consistent with a diagnosis of TTP than preeclampsia or HUS. The main distinctive features of HUS are its tendency to occur in the postpartum period and the severity of the associated renal failure. An ADAMTS13 activity $<$5% can also be used as a diagnostic test for TTP and help differentiate it from preeclampsia or aHUS (441,457).

Renal biopsy may be appropriate if there has been no improvement in renal function within 1 to 3 weeks after delivery (as would be expected in preeclampsia). However, caution is warranted if there is a persistently low platelet count.

Treatment of preeclampsia/HELLP syndrome is delivery and supportive care. More aggressive treatment is rarely indicated. Management of preeclampsia is further discussed in the Management of Preeclampsia section of this chapter. The treatment of TMA in pregnancy includes plasma infusion/exchange as would be used in nonpregnant patients with these conditions (442,443). Eculizumab has been used successfully for the treatment of aHUS during pregnancy (442,458).

Acute Fatty Liver of Pregnancy

failure. Women with AFLP often have features that are indistinguishable from either preeclampsia or HELLP syndrome (459). Characteristic laboratory findings include markedly elevated AST and ALT, elevated bilirubin, low fibrinogen, prolonged partial thromboplastin time, hypoglycemia, anemia, and thrombocytopenia (460).

AKI is a well-described complication of AFLP. A potential mechanism for AKI is the inhibition of renal fat β-oxidation due to AFLP-induced mitochondrial dysfunction leading to microvesicular fat deposition in the kidney (461,462).

Maternal mortality of AFLP is 12.5% and up to 33% in smaller series, whereas perinatal mortality is 7% to 15% (460,463). AFLP is a highly morbid and potentially lethal disease; early recognition and urgent delivery are required to prevent adverse outcomes.

URINARY TRACT INFECTIONS DURING PREGNANCY

The dilation and stasis in the urinary tract associated with pregnancy increase the risk of developing UTIs. Pregnant women with asymptomatic bacteriuria are at an increased risk of developing pyelonephritis (especially when they have a prior history of UTIs), sepsis, and AKI (464,465). Routine screening for bacteriuria in pregnancy is debated as the incidence of pyelonephritis in asymptomatic women with bacteriuria is only 2.4% (466), and has mostly been deferred to provider preference and patient's individual risk for UTI. Antibiotic therapy for asymptomatic bacteriuria decreases the risk for pyelonephritis and improves pregnancy outcomes (by decreasing SGA and preterm birth) (467). Asymptomatic bacteriuria should be treated in pregnancy, preferably with a 4- to 7-day course of oral antibiotics (468). Women who have flank pain should be treated empirically for pyelonephritis with intravenous antibiotics. After 48 hours of intravenous antibiotics, the physician may consider switching to an oral regimen, if the patient is afebrile and stable (469,470).

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CHAPTER 14

Proteinuria and Nephrotic Syndrome

Shubha Ananthakrishnan and George A. Kaysen

The ability of the kidney to retain plasma proteins is essential for life. Normal serum protein concentration is of the order of 80 mg/mL, whereas urine contains ≤150 mg of protein per day. Additionally, only a small fraction of urinary proteins is of serum origin, suggesting that nearly all plasma proteins are either restricted from filtration or effectively reabsorbed by the renal tubules and podocytes once they pass the glomerular filtration barrier. Detection of abnormal amounts or types of protein in the urine is frequently the first sign of significant renal or systemic disease. The presence of abnormal amounts of protein in the urine may reflect (a) a defect in the glomerular barrier that allows abnormal amounts of proteins of intermediate molecular weight to enter Bowman space (glomerular proteinuria), (b) diseases resulting in the inability of the kidney to reabsorb normally proteins presented to the renal tubules (tubular proteinuria), and (c) the overproduction of plasma proteins capable of passing the normal glomerular basement membrane (GBM) so that they enter tubular fluid in quantities that exceed the capacity of the normal proximal tubule to reabsorb them (overflow proteinuria). These changes, especially in the case of trans-glomerular protein loss, alter plasma protein composition in ways that favor thrombosis, vascular disease, and infectious complications.

Glomerular Mechanisms of Proteinuria

The glomerular filtration barrier consists of three layers: the endothelial layer, the GBM, and the epithelial cell layer (Fig. 14-1). It is useful to consider each layer separately, given recent developments in form and function (1).

THE ENDOTHELIAL CELL LAYER

The fenestrated endothelial layer has pores ranging in size of 60 to 80 nm allowing free passage of fluids as well as albumin (2,3). The glycocalyx layer overlying the luminal side of the endothelial layer then provides an important filtration barrier. The glycocalyx layer, a carbohydrate-rich gel with associated proteins (proteoglycans, glycoproteins, and sialic acid) forms a sieve-like structure hindering passage of large molecules (4–6). Also adsorbed into this layer are circulating and secreted molecules forming the endothelial surface layer (ESL) (7) (Fig. 14-2). Among the molecules in the ESL, hyaluronan and heparan sulfate are expressed abundantly, forming a mesh, effectively causing size, charge selectivity, and steric hindrance. Studies have explored several factors that potentially cause damage to this ESL. In a study using immortalized human glomerular endothelial cells, exposure to high glucose in vitro caused damage to the glycocalyx layer, in particular a reduction in heparan sulfate and permitted increased passage of albumin across the endothelial layer (8). The same group also showed that the glycocalyx layer could also be damaged by reactive oxygen species thus providing a mechanism for proteinuria in conditions associated with high oxidative stress (9). In summary, the glycocalyx and the proteins bound to it likely form a size barrier and the polyanions (such as heparan sulfate) repel negatively charged albumin (10).

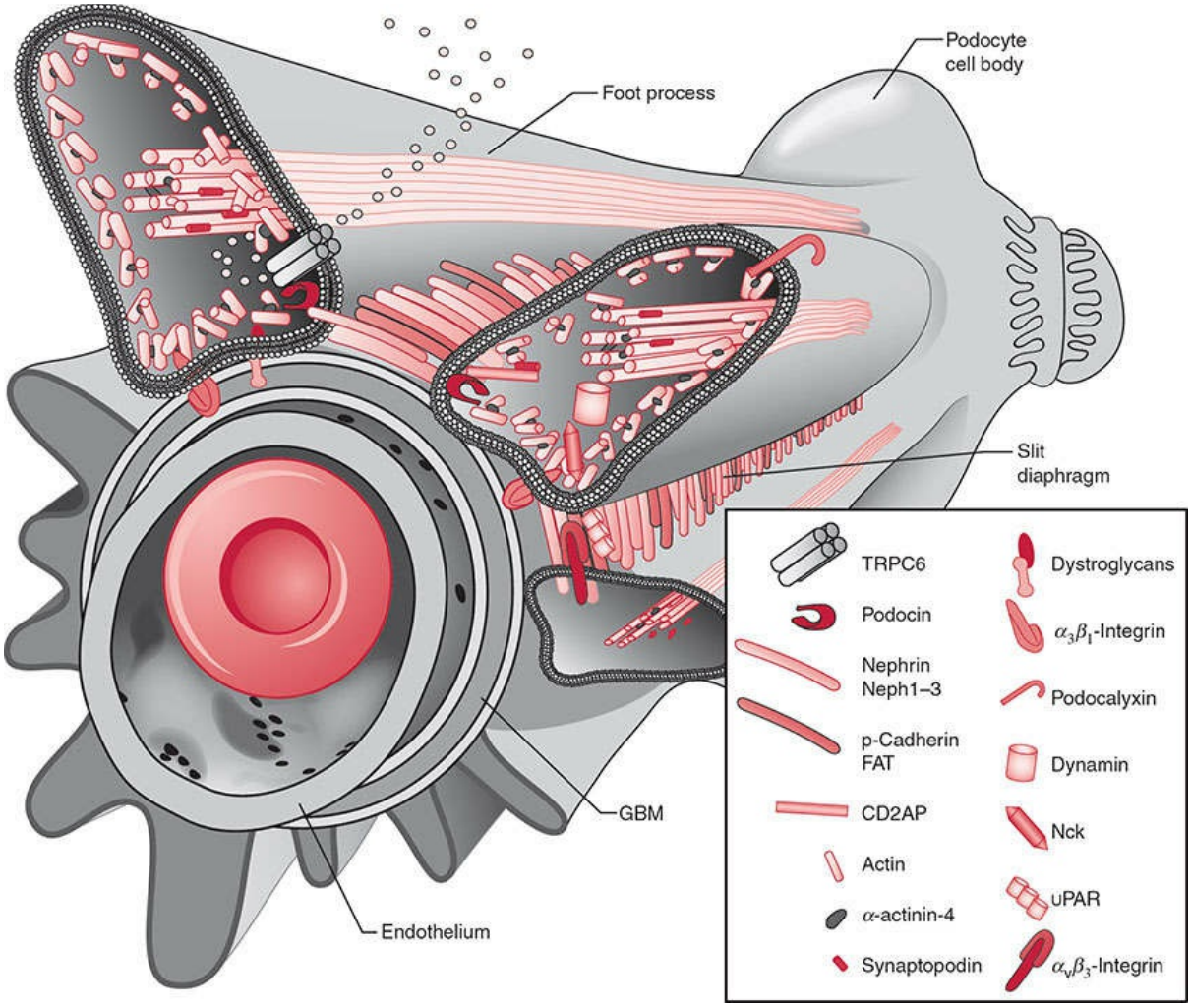

Figure 14–1 Schematic representation of the glomerular filtration barrier. The inner surface of glomerular capillaries is decorated by a fenestrated endothelium. The glomerular basement membrane (GBM) is formed by the underlying endothelial cells and overlying visceral epithelial cells (podocytes). Podocytes cover the outer aspects of the GBM with foot processes, thin extensions with a mean width of approximately 600 nm. Podocytes are anchored in the GBM via a_3b_1-integrins and *a*-/*b*-dystroglycans. The space between neighboring foot processes is filled by the glomerular slit diaphragm, a zipper-like structure formed by a number of podocyte proteins, including nephrin, neph1–3, p-cadherin, and FAT. (Republished with permission of American Society of Nephrology from Möller CC, Flesche J, Reiser J. Sensitizing the slit diaphragm with TRPC6 ion channels. *J Am Soc Nephrol*. 2009;20(5):950–953; permission conveyed through Copyright Clearance Center, Inc.)

THE GLOMERULAR BASEMENT MEMBRANE

The GBM (Fig. 14-3) has a gel-like structure, in which proteins cross mainly by diffusion (11). The basement membrane forms the structural skeleton to which endothelial and epithelial cells anchor. It is mostly composed of type 4 collagen, laminin, agrin, proteoglycans and nidogen,

of which the type 4 collagen likely contributes most to the tensile strength but only plays a minor role in filtration selectivity (1). Laminins, on the other hand, are important proteins that regulate basement membrane permeability, as β_2 laminin-deficient mice exhibit massive proteinuria (12). Heparan sulfate proteoglycans in the GBM with negatively charged side chains were thought to contribute to the charge selectivity of the filtration barrier. Experiments using glycosaminoglycan (GAG) degrading enzymes were shown to increase glomerular permeability, related to degradation of heparan sulfate (13). However, as seen above, the glycocalyx layer also possesses GAGs and earlier experiments probably were unable to differentiate the relative contributions of each of these layers to the total charge selectivity of the filtration barrier. The GBM does provide a relatively small degree of size selective barrier to filtration. In an elegant experiment where cell-free glomerular skeleton, composed almost entirely of GBM, was used to study diffusion of Ficoll (a neutral substance), the contribution of GBM to diffusional resistance was found to be around 13% to 26% of total, increasing with size (14).

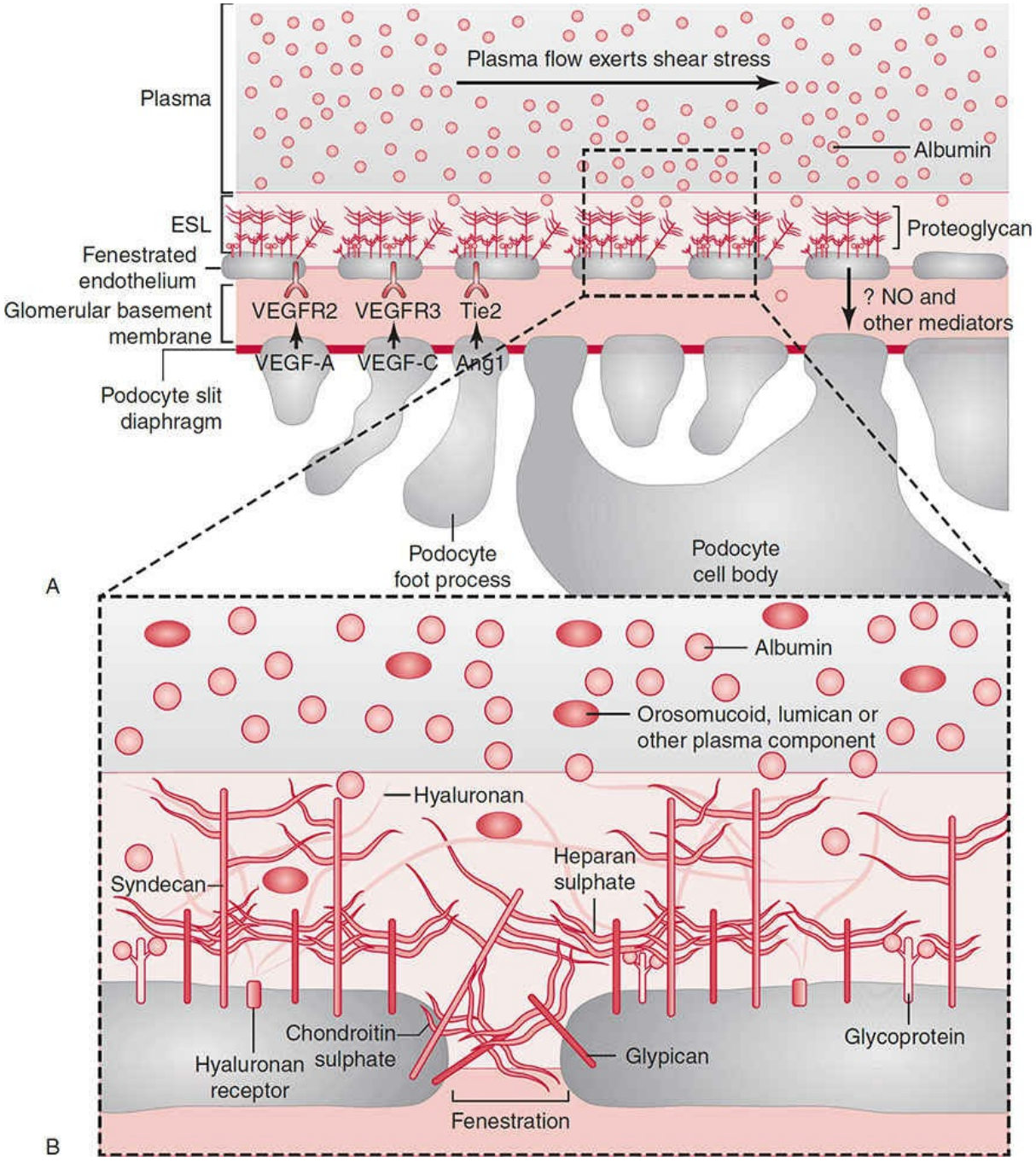

Figure 14–2 (**A**) The ESL (endothelial surface layer) comprises the cell surface–anchored glycocalyx and adsorbed plasma constituents, and covers the luminal surface of the endothelium, extending over and into the fenestrae. The ESL forms the first barrier to albumin passage across the glomerular filtration barrier and ensures that albumin is largely confined to the capillary lumen. (**B**) Detail of the ESL showing its heterogeneous structure. The cell surface–anchored proteoglycan core proteins include glypicans and syndecans, which have anionic glycosaminoglycan side chains. Glypicans have heparan sulfate chains, whereas syndecans have chondroitin sulfate chains. Hyaluronan, which is often present in very long chains, binds to cell-surface receptors including CD44 and may also be more loosely adsorbed onto cell-surface–anchored molecules along with other plasma components. Glycoproteins may have short carbohydrate side chains and terminal sialic acid residues. The ESL, therefore, forms a

negatively charged barrier to the passage of albumin. (Reprinted by permission from Macmillan Publishers Ltd: *Nat Rev Nephrol*. Satchell S. The role of the glomerular endothelium in albumin handling. 2013;9(12):717–725)

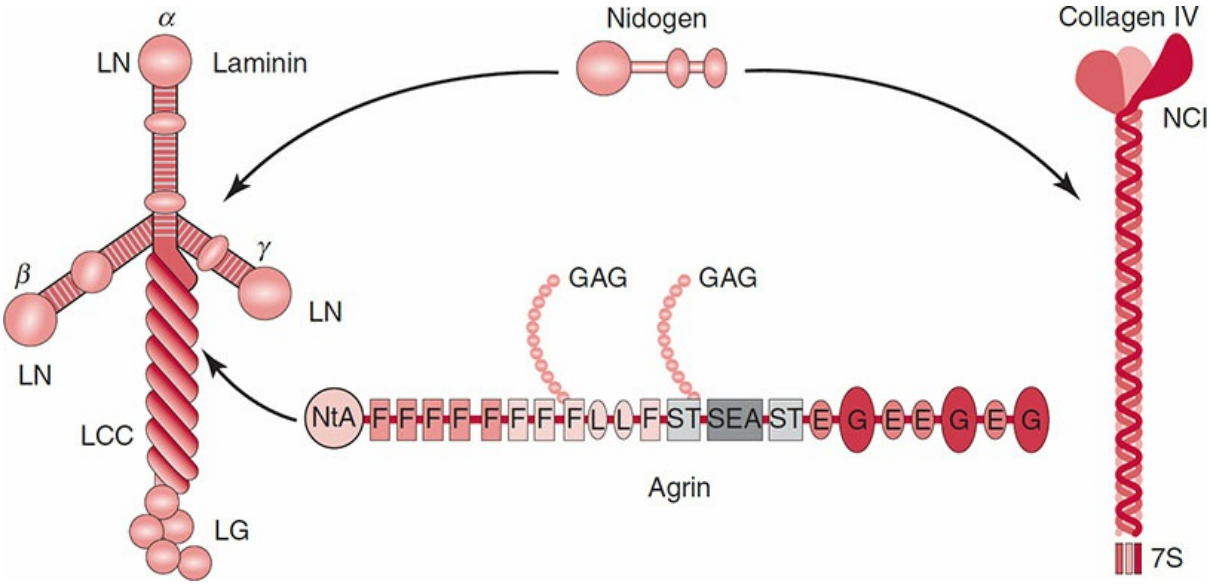

Figure 14–3 The major components of basement membranes: laminin, type IV collagen, nidogen, and heparan sulfate proteoglycan (agrin is shown because of its prevalence in the GBM, though perlecan is more widely found in basement membranes). Collagen IV is a triple helical protein with *C*-terminal noncollagenous domains (NCI) and *N*-terminal 7S domains; these are important in network formation. Laminin *a*, *b*, and *g* chains assemble with each other via the laminin coiled-coil (LCC) domain. Laminin *N*-terminal (LN) domains are involved in polymerization of trimers, which initiates basement membrane formation. The *C*-terminal laminin globular (LG) domain contains binding sites for cell-surface receptors. Agrin, a modular protein containing glycosaminoglycan (GAG) side chains, binds to the laminin long arm via the g_1 chain, whereas nidogen binds to the short arm of laminin g_1 as well as to collagen IV. (Reprinted from Miner JH. The glomerular basement membrane. *Exp Cell Res*. 2012;318(9):973–978, with permission from Elsevier.)

THE PODOCYTE

The podocyte is a specialized cell that gives off interdigitating processes known as foot processes that surround the capillaries (15) (Fig. 14-4). The basal surfaces of the podocytes adhere to the GBM and the interdigitating processes also form cell–cell junctions known as the slit diaphragms. There have been numerous developments in the identification of key proteins involved in the regulation and maintenance of the foot processes and the slit diaphragm. Integrins (especially $\alpha_3\beta_1$ integrin) are one of the main proteins that facilitate attachment of the basal surface of the podocyte with the GBM (Fig. 14-5). Other proteins spanning the podocyte and GBM

include dystroglycans and tetraspanins. At the apical surface is podocalyxin. These transmembrane proteins attach internally to the actin cytoskeleton (1). Of note is also the urokinase-type plasminogen receptor (uPAR) in the basal surface that has a circulating form—soluble uPAR (SuPAR) that is cleaved from the membrane. SuPAR has garnered interest in potentially being a marker of progressive decline in kidney function (16). The slit diaphragm, which is the space between adjacent foot processes, is a zipper-like structure formed by nephrin, neph1-3, p-cadherin, and FAT (17). Nephrin gene mutation is associated with congenital nephrotic syndrome of the Finnish type (18). Podocin is another important slit diaphragm protein that interacts with nephrin. Podocin plays a crucial role in maintaining the filtration barrier. Mutation of podocin is associated with familial steroid-resistant nephrotic syndromes (19).

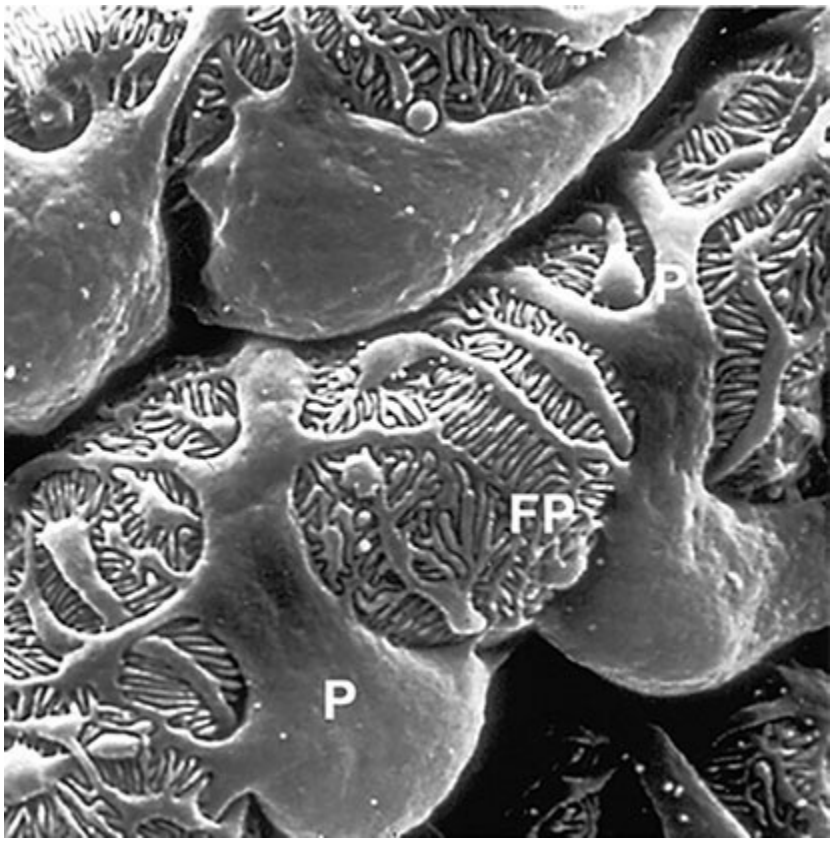

Figure 14–4 The podocyte (P): shown here as a specialized cell that gives off interdigitating processes known as foot processes (FP) that surround the capillaries. (Republished with permission of The Company of Biologists Ltd. from Quaggin SE, Kreidberg JA. Development of the renal glomerulus: good neighbors and good fences. *Development*. 2008;135(4):609–620; permission conveyed through Copyright Clearance Center, Inc., with permission.)

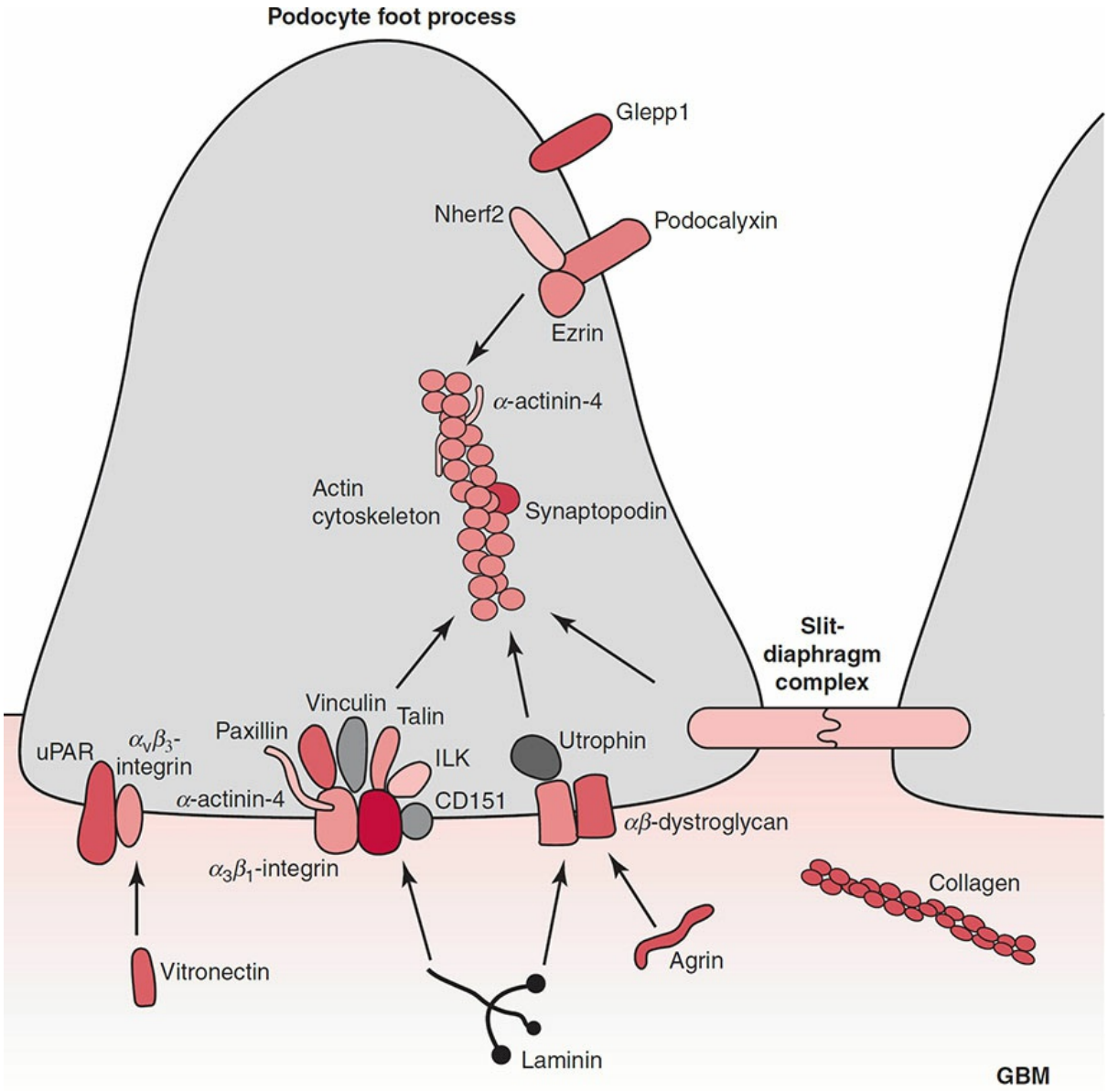

Figure 14–5 Schematic picture of podocyte foot processes. Foot processes are basally anchored to the components of the GBM via a_3b_1 integrin, tetraspanin CD151, and *ab* dystroglycan. These transmembrane proteins are linked to the actin cytoskeleton via several adaptor proteins. uPAR receptor is also found at the basal surface of foot processes where it probably mediates its actions through a_vb_3-integrin. Vitronectin, the extracellular ligand of a_vb_3-integrin, is induced during proteinuria and activates uPAR signaling in podocytes. The slit diaphragm protein complex is linked to actin cytoskeleton. Apical surface of podocytes contain podocalyxin and Glepp1. Podocalyxin is connected to actin via adapter proteins. The actin cytoskeleton of foot processes contains actin-associated proteins *a*-actinin-4 and synaptopodin, and interconnects three plasma membrane domains of foot processes together. (Reprinted from Patrakka J, Tryggvason K. Molecular make-up of the glomerular filtration barrier. *Biochem Biophys Res Commun*. 2010;396(1):164–169, with permission from Elsevier.)

Podocyte effacement is a common manifestation of proteinuric

nephropathies. Is podocyte effacement an effect or a causative factor of proteinuria? When podocytes are faced with glomerular proteinuria, there is excessive uptake of albumin and immunoglobulin G (IgG) in the podocyte through specific receptors. This can cause redistribution of F-actin fibers in the cytoskeleton, resulting in damaged podocytes (20). Hyperglycemia-induced glycated albumin could potentially also be involved in podocyte injury and damage (10). In a recent mice experiment, when endothelium-specific hyaluron synthase was missing, the endothelial glycocalyx later was lost, the mice developed albuminuria, and within weeks developed podocyte injury and glomerulosclerosis (21). Studies of drugs that stabilize the glycocalyx layer, such as sulodexide, however, have failed to demonstrate beneficial effects in progression of diabetic kidney disease (22,23).

It has been well described that in certain conditions, proteinuria occurs with no foot process effacement, such as in nephrin knockout mice, causing massive proteinuria (24). Experimental vascular endothelial growth factor blockade in mice also causes proteinuria without foot process effacement, with endothelial changes such as endotheliosis and with vacuolization, similar to that seen in preeclampsia (25). What seems to be clear is that damage to any of the three components of the glomerular filtration barrier can induce proteinuria. Sustained proteinuria from damage of any of the layers then could cause eventual foot process effacement (24).

As can be seen from the description of the endothelial layer and the basement membrane, they provide relatively less restriction to filtration of albumin, although the glycocalyx layer provides steric hindrance. The filtration slit diaphragm then has an important contribution to restriction of filtration of macromolecules (26). Total permeability is a function of resistance of each of the three layers. Change in one component of the filtration barrier affects the overall permeability by the same proportion (7,27).

It is traditionally held that the glomerulus is a charge and size selective barrier. Neutral and negatively charged dextrans are filtered by the glomerulus, but are neither reabsorbed nor catabolized by the renal tubule, and thus serve as probes of glomerular size and charge selectivity (28). Neutral dextrans and other nonmetabolized organic molecules are restricted from the urine on the basis of size and shape, but not of charge (29). Negatively charged molecules are more restricted than neutral molecules (30) because of electrostatic interaction with the glomerular filtration barrier. However, more recent in vivo studies have challenged

this concept of a charge selective barrier, despite the presence of negatively charged heparan sulfate proteoglycans (31,32). Figure 14-6 shows the relative renal clearance of neutral dextrans of increasing molecular radius. The curves bearing open symbols represent the clearance of dextrans by the normal human kidney. As the radius of dextrans increases, their clearance relative to inulin, and therefore to water, decreases.

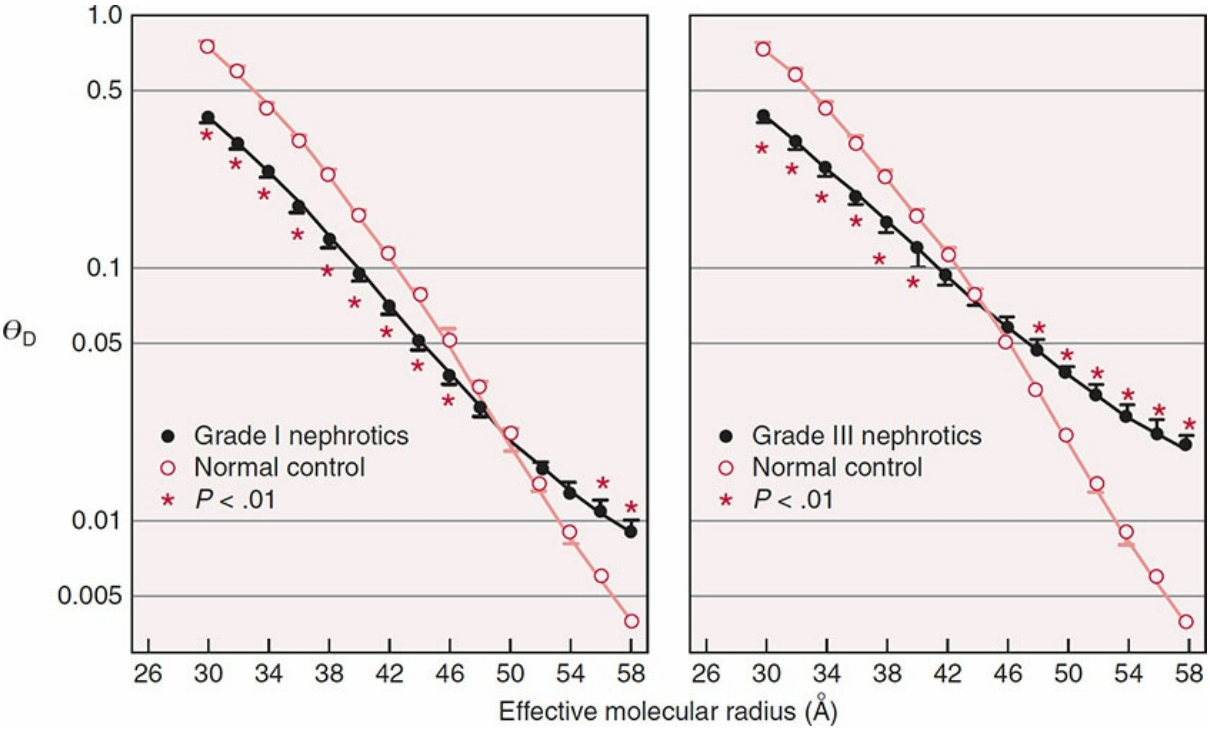

Figure 14–6 Fractional dextran clearances plotted as a function of effective molecular radius. Data from normal subjects are represented by curves bearing *open symbols* in both panels. Data from patients with the nephrotic syndrome are represented by *curves* bearing *closed symbols*. Dextran sieving curves from patients with mild renal damage are represented in the **left panel** and sieving curves from patients with severe glomerular lesions are represented in the **right panel**. All results are expressed as means ± SE. Statistical differences between control and experimental values are connoted by *asterisk* and reflect a difference at $P < .01$. (From Deen WM, Bridges CR, Brenner BM, et al. Heterosporous model of glomerular size selectivity: application to normal and nephrotic humans. *Am J Physiol*. 249:F374–F389, 1985, with permission.)

One may depict the normal glomerular filtration barrier as being occupied by a series of pores that allow the unrestricted passage of low-molecular-weight solutes and progressively restrict the passage of molecules of greater molecular radius. Previous studies using solute clearance techniques determined that the vast majority of the surface is represented as covered by many pores of similar size, small enough to

restrict the passage of large or intermediate-molecular-weight proteins, but freely permeable to water and small-molecular-weight peptides and carbohydrate polymers. These pores were estimated to have a radius of between 5.1 and 5.7 nM. A second, much smaller population of much larger pores was also represented on this hypothetical glomerular filtration barrier. These pores were thought to be relatively unselective to molecules of intermediate size and form a shunt pathway that allowed proteins to pass into the ultrafiltrate unencumbered (33). Using other techniques such as electron microscopy, Rodewald and Karnowsky (34) first described the filtration slit diaphragms as having fairly uniform slit sizes around 30 to 45 nm. More recently, using advanced scanning electron microscopy techniques in rats, the filtration slit diaphragm pores were described to have both circular and ellipsoidal shapes, and were log-normally distributed with a mean pore radius of 12.1 nm. Proteinuric rats had more large pores (35). Most diseases that cause the nephrotic syndrome in man primarily cause a loss of glomerular size selectivity without a loss of charge selectivity. The quality of proteins that appear in urine however also support a sieving mechanism that is more selective than size alone (36) (Specifically the fatty acid content of albumin that appears in urine is significantly less than the fatty acid content of albumin that is retained.) The significance of this retention of albumin that is highly saturated with free fatty acids (FFAs) plays a significant role in generating the hypertriglyceridemia associated with the nephrotic syndrome by promoting the secretion of angiopoietin-like 4 (Angptl4), a protein that inhibits lipoprotein lipase (LPL) from skeletal muscle, adipose tissue, and heart (37).

RENAL HANDLING OF PROTEINS

Using micropuncture studies in rats, it is estimated that the concentration of albumin in the proximal tubular fluid is around 20 to 30 *m*g/mL (38,39). The small amounts of filtered albumin are then reclaimed in the proximal tubule via the megalin–cubilin-mediated endocytic pathway (40,41). The albumin resorbed by the proximal tubular cells then undergoes either degradation or reclamation back into the capillaries (42). Recent studies indicate that the proximal tubule might also regulate albumin reclamation in response to plasma albumin levels. In a study by Wagner et al., albumin loading in rats led to reduced proximal tubular uptake of albumin, leading to increased albumin losses in urine. Conversely, in the face of increased glomerular proteinuria, and hence development of hypoalbuminemia, the

proximal tubule avidly uptakes albumin via the reclamation pathway, thereby minimizing urinary albumin loss (42,43) (Fig. 14.7). These recent findings of more complex renal handling of proteins could eventually improve our understanding of renal disease progression.

FACTORS AFFECTING GLOMERULAR PERMEABILITY

Alterations in glomerular permeability can occur quickly, may be transient, and are hemodynamically (44) or hormonally mediated. Among various hormones affecting glomerular permeability, probably the most important include the effects of angiotensin and hence, clinically, the effects of angiotensin blockade.

Infusion of angiotensin II into the renal artery promptly induces a significant proteinuria, that is abolished by pretreatment with angiotensin II (Ang II) receptor antagonist (45). The beneficial effects of angiotensin--converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) were initially considered to be caused by reduced Ang II, thereby reducing intraglomerular pressure reduction of proteinuria. ACEI and ARBs did not cause changes in the filtration slit radius or pore distribution (46).

In experimental Heymann nephritis (membranous nephropathy being the human counterpart), there was downregulation of nephrin gene expression and nephrin staining; ACEI or ARB was able to completely block this effect on nephrin downregulation, suggesting that the renoprotective effect of these drugs may be mediated by salutary effects on nephrin assembly (47).

Thromboxane synthesis also may play a role in the development of proteinuria in some forms of renal diseases (48). Both cyclooxygenase inhibitors and ACEIs reduce proteinuria in patients with the nephrotic syndrome in part by reducing the fraction of glomerular filtrate that passes through the large pores. Nitric oxide (NO) is a potent vasodilator released by vascular endothelial cells and macrophages, is derived from the guanidino group on arginine, and plays a role in the regulation of renal blood flow in normal and pathologic states (49,50). Baylis reported that inhibition of NO caused both glomerular hypertension and proteinuria in normal rats (51). It is not known at this time whether NO modulates proteinuria in nephrotic syndrome in patients.

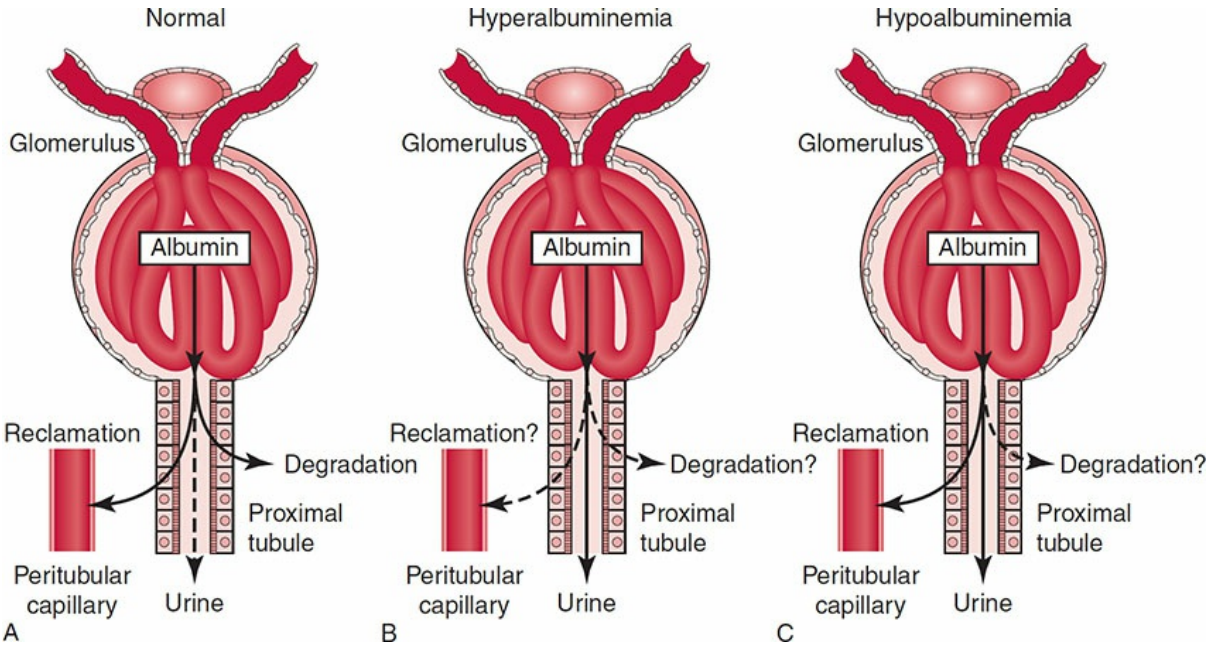

Figure 14–7 (**A**) Under normal conditions plasma albumin is filtered across the glomerulus and is taken up by the proximal tubules to be reclaimed or degraded with only very small amounts of intact albumin excreted in urine. (**B**) The glomerular sieving coefficient does not change markedly in response to increased levels of plasma albumin (hyperalbuminemia); however, the proximal tubule reduces uptake of filtered albumin and excess albumin is lost in the urine. (**C**) When the glomerular barrier is compromised (hypoalbuminemia), albumin is lost into the tubular lumen. The proximal tubule responds by increasing albumin uptake, most likely via upregulation of the reclamation pathway, thereby minimizing urinary albumin loss. (Reprinted from Patrakka J, Tryggvason K. Molecular make-up of the glomerular filtration barrier. *Biochem Biophys Res Commun*. 2010;396(1):164–169, with permission from Elsevier.)

SELECTIVITY OF GLOMERULAR PROTEINURIA

Urine protein electrophoresis patterns have been used in the past to distinguish between different diseases causing glomerular proteinuria. Minimal change nephrotic syndrome classically has been regarded as causing “selective” proteinuria characterized by a predominance of albumin in comparison with other proteins of intermediate molecular weight. It was believed that minimal change nephrotic syndrome resulted from loss of charge selectivity, so highly negatively charged albumin was lost in the urine because albumin was restricted on the basis of charge alone, whereas other larger but more neutrally charged proteins were retained. However, minimal change nephrotic syndrome also is characterized by altered size selectivity, similar to other diseases that cause nephrotic syndrome (52). Many disease entities that can cause proteinuria may cause selective or nonselective proteinuria. The urinary protein

electrophoretic pattern encountered in these diseases is determined by the relative fraction of glomerular ultrafiltrate that passes through these large pores.

Renal biopsy has long replaced measurement of the relative concentrations of different proteins in the urine for determination of glomerular pathology. Urinary protein electrophoresis is useful for distinguishing between tubular proteinuria, overflow proteinuria, and glomerular proteinuria but has little utility for distinguishing between diseases of glomerular origin.

Tubular Proteinuria

Tubular proteinuria occurs from a failure of the proximal tubule to reabsorb proteins in the normal glomerular filtrate. Quantitatively, these proteins are generally in the range of greater than 150 mg/day but less than 1.5 g/day. The majority of the protein found in the urine in patients with tubular proteinuria is of lower molecular weight, such as β_2 microglobulin (MW 11.6 kDa) and α_1 microglobulin (MW 31 kDa). Albumin with a molecular weight of 69 kDa is only modestly increased in tubular proteinuria, when the glomerular filtration barrier is intact. In its most severe form, proximal tubular dysfunction is characterized by the "Fanconi syndrome" where there is an inability to reabsorb glucose, amino acids, uric acid, phosphate, bicarbonate, and other normal components of proximal tubular fluid in addition to proteins. As a consequence, the Fanconi syndrome causes a nonanion gap metabolic acidosis, hypouricemia, hypophosphatemia, aminoaciduria, and glycosuria in addition to proteinuria. In addition, high urinary concentrations of molecules such as parathyroid hormone, insulin, insulin-like growth hormone (IGF-1) and chemokine monocyte chemoattractant protein-1 (MCP-1) were found in patients with Fanconi syndrome, which may potentially be involved in progressive interstitial fibrosis and renal failure (53,54). Urinary retinol binding protein (RBP) might also be valuable in diagnosis of tubular proteinuria (55) and urinary RBP: creatinine ratio has been suggested by some as a useful screening test for tubular proteinuria (56,57). The Fanconi syndrome may result from inherited metabolic disorders such as Dent's disease, cadmium exposure, light chain-associated diseases such as myeloma, amyloidosis, etc. In clinical practice, probably the most commonly encountered setting for Fanconi syndrome is

medications such as tenofovir (56). Early detection of proximal tubular defects by medications such as tenofovir is important as withdrawal of such agents have been shown to have beneficial effects on estimated glomerular filtration rate (GFR) (58). Table 14-1 lists some causes of tubular proteinuria.

Table 14–1 Disorders Causing Impaired Renal Reabsorption of Filtered Proteins at Normal Filtered Loads

Congenital disorders
Fanconi syndrome
Hereditary
Cystinosis
Wilson disease
Heritable fructose intolerance
Oxalosis
Hereditary tyrosinemia (267)
Glycogen storage diseases
Galactosemia
Dent disease
Lowe syndrome
Acquired
Heavy metal poisoning
Tenofovir
Multiple myeloma
Amyloidosis
Vitamin D intoxication
Bartter syndrome
Familial asymptomatic tubular proteinuria
Oculocerebrorenal dystrophy
Renal tubular acidosis
Renal dysplasia
Renal cystic disorders (polycystic kidney disease)
Systemic disease
Hereditary
Galactosemia
Glycogen storage disease
Acquired
Balkan nephropathy
Sarcoidosis

Systemic lupus erythematosus
Acute renal disease
Acute tubular necrosis
Renal infarction
Transplant rejection
Infectious disease
Pyelonephritis
Viral or bacterial associated interstitial nephritis
Drugs and toxins
Acute hypersensitivity interstitial nephritis (penicillins, cephalosporins, sulfonamides)
Aminoglycoside toxicity
Analgesic nephropathy
Cyclosporin toxicity
Cd, Pb, As, Hg, ethylene glycol, CC14
Vitamin D intoxication

Overflow Proteinuria

An increase in the filtered load of certain proteins such as light chains, results in overflow proteinuria. Megalin and cubilin in the proximal tubule which mediate albumin reabsorption also participate in reabsorption of light chains (59). Overflow proteinuria occurs when these receptors are overwhelmed by the large filtered load of proteins such as light chains in myeloma. Figure 14-8 shows the result of protein electrophoresis of urine from a patient with this disorder. There is a large quantity of light chain protein in the urine (a so-called “spike”). Some myeloma light chain proteins are quite nephrotoxic, depending on their isoelectric points (pKi) and other factors. Myeloma light chain proteins with a pKi of around 5 generally are most toxic, in part because of their reduced solubility in the acid milieu of the renal papilla (60). Benign causes of overflow proteinuria include myoglobinuria in patients with rhabdomyolysis (61).

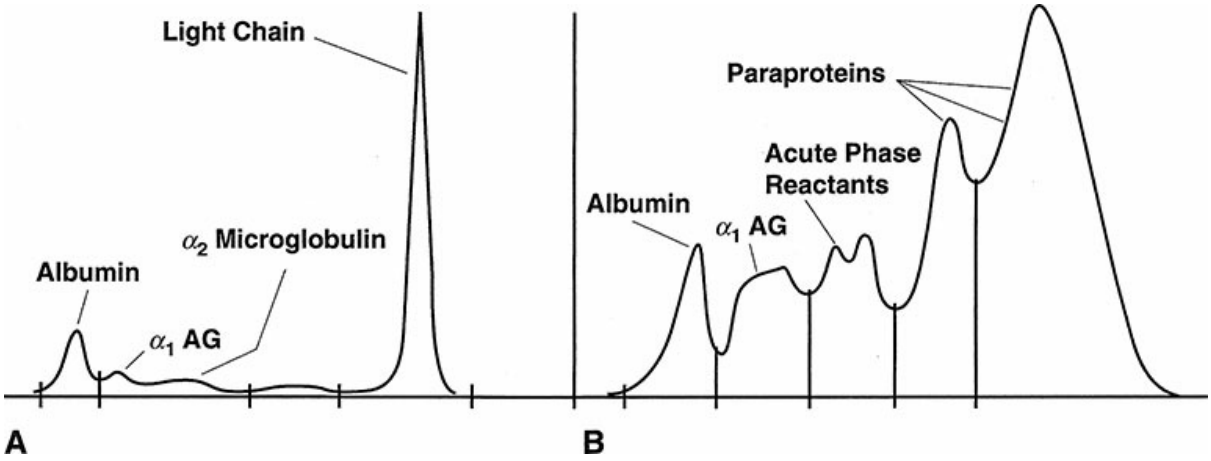

Figure 14–8 Overflow proteinuria: scan of electrophoresis of protein from the urine of a patient with multiple myeloma **(panel A)** and a patient with AIDS **(panel B)**. Note the predominance of protein in a single band in the urine of the patient with multiple myeloma. This is caused by the overflow of a homogeneous cationic immunoglobulin fragment (*light chains*). In the patient with AIDS, urinary protein is composed of a heterogeneous mixtures of acute-phase reactant proteins and polyclonal immunoglobulin fragments. Protein concentration is 16 mg/dL in the urine in **panel A**. Albumin represents 13.4% of total protein, a_1 AG represents 7.6%, a_2 microglobulin represents 8.1%, *b* globulins 5.3%, and *g* globulin is 65.6% of total urinary protein. Protein concentration is 220 mg/dL in the urine in **panel B**. Albumin represents 7.6% of total protein, a_1 AG represents 11.7%, a_2 microglobulin represents 13.3%, *b* globulins 18.3%, and *g* globulin is 49.2% of total urinary protein.

Ig appearance in the urine, however, does not necessarily indicate malignant disease. Monoclonal gammopathy of undetermined significance may never progress to malignant transformation (62), although the cells responsible for generating this low amount of Ig ultimately may undergo malignant transformation, given sufficient time (63) with a rate of onset of frank multiple myeloma of approximately 1%/year (64).

A modest increase in urinary protein excretion can occur in patients during acute inflammatory conditions, such as in patients with human immunodeficiency virus (HIV) infection, after trauma, or as a consequence of severe infection. This is a consequence of increased excretion of a number of low-molecular-weight proteins produced in response to stress, Ig, and acute-phase reactant proteins. Their filtration is increased beyond the tubular capacity for their reabsorption, and they spill into the urine and should be distinguished from glomerular proteinuria, which also can be caused by HIV infection. Acute-phase reactant proteins of relatively low molecular weight appear in the urine as well as polyclonal Ig fragments (paraproteins). These represent the filtration of a variety of polyclonal Ig fragments produced in excess as a result of HIV infection (65,66). The

most common causes of overflow proteinuria are listed in Table 14-2.

Table 14–2 Causes of Overflow Proteinuria

Monoclonal gammopathy of undetermined significance
Multiple myeloma
Monocytic and myelomonocytic leukemia
Hemoglobinuria
Myoglobinuria
Systemic inflammatory processes
Trauma
Sepsis
HIV infection

Benign or Physiologic Causes of Proteinuria

Transient or self-limiting proteinuria can occur under physiologic states of fever, exercise, etc., possibly mediated by alterations in glomerular permeability and by inhibition of protein reabsorption (67,68). Postural or orthostatic proteinuria is occurrence of proteinuria in the upright position, which disappears in the supine position. This condition is most often seen in the young and adolescent population and has an excellent overall prognosis as it resolves over time (69–71). Orthostatic proteinuria is diagnosed using a split urine collection. Detailed protocols are available for testing for this condition (70).

Methods for Measuring Proteinuria

There are several qualitative and quantitative tests available to measure urinary protein excretion. Clinic screening for the presence of proteinuria is generally performed with the use of the urinary dipstick. The reactive portion of the stick is coated with a buffered indicator that changes color in the presence of protein. Urinary dipstick results are semiquantitative and are better utilized as a screening tool for the presence of protein in the urine and a rough guide to urine protein concentration. The standard urinary dipstick measures albumin concentration via a colorimetric reaction between albumin and tetrabromophenol. Use of this dye-binding

technique has a number of limitations including that the indicator is more sensitive to albumin than other plasma proteins, and thus is a poor marker for tubular and overflow proteinuria. Moreover, the urinary dipsticks usually become positive when protein excretion rates exceed 300 to 500 mg/day, thus it is not a good screening tool for detecting very low-grade proteinuria or microalbuminuria (72). False-positive urinary dipstick results have been reported when patients have been administered radiocontrast agents (73).

The classic method of quantifying the amount of proteinuria is a timed 24-hour urine collection. Because of frequent collection errors and unreliability, this common technique of quantifying urine protein excretion has come under criticism. Random or first morning single-specimen (spot) collection with measurement of protein to creatinine concentration (UPC) ratios has been advocated as a more reliable way to assess the degree of proteinuria (74). There is good correlation between the first morning spot urine specimen measurement of the UPC ratio and a timed 24-hour urine collection for protein excretion determination. Similarly, a spot urine albumin to creatinine (UAC) ratio is a useful means to assess the degree of urinary albumin excretion for a 24-hour period. Caution is advised in the utilization of the UPC and UAC ratios in patients with extremes of muscle mass. For example, in patients with very low muscle mass, the denominator is quite low, thus falsely overestimating the UPC and hence the 24 hour urine protein excretion.

The standard method for assessing and quantifying urinary albumin excretion is based on the binding of the pH-sensitive bromcresol green dye by albumin. When bromcresol green is bound to albumin, the dissociation constant of the dye changes so that it is in its ionized form at all physiologic urinary pH values and turns green. This method does not detect other urinary proteins well and is relatively insensitive, the lower limit of detection being slightly less than 30 mg/dL. If total urine volume is 1 L, urinary albumin excretion will reach the threshold of detection with this method at slightly less than 300 mg/day. Because clinically important albuminuria occurs well below this value (>22 mg/day), more sensitive, immunologically based assays using nephelometric methods are employed to detect small amounts of albumin (microalbuminuria) in the urine that may reveal the presence of clinically significant renal diseases, such as diabetic nephropathy, in their early stages.

When tubular proteinuria is suspected, urine electrophoresis is a useful initial test. Electrophoretic methods are useful in initial evaluation of urinary protein to determine whether the pattern of protein excretion is

most compatible with that resulting from a tubular lesion, overflow of abnormal proteins into the urine, or glomerular pathology. Subsequently, more specific tests such as for β_2 microglobulin or RBP can be measured in commercial laboratories using chemiluminescent assays or nephelometry.

MYOGLOBINURIA

Damage to striated muscle causes the appearance of myoglobin in the blood. This low-molecular-weight protein is freely filtered by the glomerulus and may appear in the urine in large quantities (75). The urine may be turbid or clear but generally is brown. After centrifugation of the urine, the supernatant tests positive for blood using the benzidine test, even in the absence of red blood cells. It is important to identify this entity for two reasons. Most significantly, myoglobinuria is an important cause of acute kidney injury (76–78). The mechanism for the acute kidney injury caused by myoglobinuria is probably multifactorial, including intense renal vasoconstriction, tubular obstruction, and, most importantly, tubular injury. Iron contained in the heme moiety of myoglobin causes direct tubular damage by acting as a Fenton (free radical) reagent (79). Therapies suggested to mitigate or attenuate myoglobin-induced acute kidney injury include vigorous parenteral fluid administration to help accelerate renal clearance of myoglobin and the addition of sodium bicarbonate and mannitol to the parenteral fluids. Alkalinizing the urine with the addition of sodium bicarbonate to parenteral fluids may help prevent the heme moiety separating from the globin component of myoglobin and thus prevent iron-induced tubular damage (80). The addition of mannitol has a number of theoretical benefits to attenuate acute kidney injury; mannitol is an osmotic diuretic and would accelerate urinary excretion of myoglobin, and it increases renal blood flow and is a scavenger of free radicals (81). However, the routine use of mannitol is not recommend in this setting given the questionable overall clinical benefit (82).

HEMOGLOBINURIA

Hemoglobinuria results from intravascular hemolysis and occurs when the capacity of haptoglobin to bind free hemoglobin is exceeded. The urine may vary from pink to black in color. Spectroscopic methods may be necessary to distinguish hemoglobinuria from myoglobinuria. It is important to identify this entity because hemoglobinuria also can cause

acute renal failure (83). As in the case of myoglobinuria, renal failure caused by hemoglobinuria (84) may be averted by mannitol infusion, hydration, and urinary alkalinization, although this approach remains controversial. Pure hemoglobin has little or no toxic effect when transfused (85), but red cell stroma alone can cause renal failure (86). Therefore, the cause of acute renal failure associated with hemoglobinuria may involve a mechanism other than tubular obstruction by filtered hemoglobin.

Hemoglobinuria may be an initial manifestation of conditions causing acute intravascular hemolysis, which may be life threatening, even in the absence of acute renal failure. These conditions include incompatible blood transfusions, arsine poisoning (87), falciparum malaria, red cell enzyme defects, immune hemolytic anemias, and acute hemolysis owing to drugs, chemicals (88), burns, hypophosphatemia (89), infections, eclampsia, or the entrance of hypotonic solutions into the blood, such as hypotonic infusions during prostatectomy. Anemia alone may cause death from many of these entities long before renal failure becomes a clinical problem.

Chronic intravascular hemolysis also may cause hemoglobinuria. Although neither severe, acute anemia nor acute renal failure develops as a consequence of chronic intravascular hemolysis, hemoglobinuria or hemosiderinuria may be the first recognizable symptom of one of several chronic disorders. Diseases responsible for chronic intravascular hemolysis include paroxysmal nocturnal hemoglobinuria (90), paroxysmal cold hemoglobinuria, march hemoglobinuria (91) (resulting from mechanical disruption of red cells during exercise—the pigment excreted also may be myoglobin), and mechanical disruption of red blood cells, owing to prosthetic heart valves (92).

Nephrotic Syndrome

Nephrotic syndrome results from alterations in the permselective characteristics of the GBM that allow increased passage of proteins of intermediate size into the urine and consists of the constellation of heavy proteinuria (≥3.5 g/day), hypoalbuminemia, hyperlipidemia, increased concentration of several high-molecular-weight proteins, reduction in the concentration of several proteins of intermediate size, and edema formation (93). Not all components of this syndrome need be present. It is not known why all manifestations of nephrotic syndrome are expressed in

some patients and not in others. Proteinuria >3.5 g/day, however, is predictive of any of several serious renal diseases listed in Table 14-3 and defines nephrotic proteinuria.

It is somewhat surprising that all of these manifestations may result from the loss of the amount of protein in half an egg of a hen. The mean value for proteinuria in a number of studies of nephrotic syndrome is about 8 g/day (94–97) but viewed in the context of normal protein intake, even this external loss is small. Although it is experimentally more difficult to quantitate the losses of tissue protein, continuous massive proteinuria causes marked muscle wasting (98) sometimes obscured by edema. How do these extensive metabolic derangements result from a relatively small amount of protein loss? What are the homeostatic adaptations that result from urinary protein loss? How do these adaptations lead to other abnormalities in plasma protein composition that also characterize the nephrotic syndrome? What other proteins are either decreased by loss or inappropriately increased in response to changes in plasma composition that contribute to morbidity? Why are urinary protein losses resistant to replacement by dietary protein augmentation and what are the effects of dietary protein augmentation both on plasma protein composition and on renal function? These are questions that are approached in the ensuing sections.

Table 14–3 Causes of Glomerular Proteinuria

Diseases confined to the kidney
Minimal change nephrotic syndrome
Membranous nephropathy
Focal segmental glomerulosclerosis
Mesangial proliferative glomerulonephritis
Acute poststreptococcal glomerulonephritis
Systemic diseases
Diabetes mellitus
Henoch–Schönlein purpura
Systemic lupus erythematosus
Amyloidosis
Goodpasture syndrome
ANCA vasculitis
Hepatitis C and hepatitis B
Hereditary disorders
Congenital nephrotic syndrome

Hereditary nephritis (Alport syndrome)
Partial lipodystrophy

ANCA, antineutrophil cytoplasmic antibody.

ALBUMIN METABOLISM IN NEPHROTIC SYNDROME

We initially will focus on albumin, as it is the most abundant protein in plasma, maintains oncotic pressure, and a reduced level of this protein is one hallmark of the nephrotic syndrome. Hypoalbuminemia has been encountered in a number of pathologic conditions, during inflammation, systemic infection or trauma, and malnutrition. The importance of focusing on albumin is (1) changes in its concentration in the nephrotic syndrome parallel changes in other important proteins that play a role in immune defense (99), hematopoiesis (100–102), and blood coagulation (103), binding proteins for important vitamins and hormones (104–106) both as a consequence of urinary loss of these proteins and as a consequence of increased synthesis of other proteins that appear to be coordinated with that of albumin in response to reduction in oncotic pressure that may not be lost in the urine because of their size and thus lead to increases in their plasma level.

Albumin also non-covalently binds a number of metabolites, including FFAs (107,108). As will be reviewed in more detail, the content of FFAs increases in albumin in patients with nephrotic range proteinuria, leading to increased delivery of FFAs to a variety of tissues leading to a reduction in LPL ultimately contributing to hypertriglyceridemia (37). Thus, albumin losses do contribute specifically to some of the metabolic alterations encountered in the nephrotic syndrome.

In the absence of external albumin loss, before the onset of albuminuria, a fixed quantity of albumin is synthesized each day and an identical quantity is destroyed by catabolism. Normal albumin turnover rate per day is between 10 and 14 g (95) which represents only about 4% of the total albumin pool; however, urinary loss in nephrotic patients represent a considerable fraction of the total quantity synthesized per day so that the capacity to replace a deficit from the reduction in the mass of a large pool is limited, especially as increasing albumin concentration by increasing the synthetic rate will be accompanied by an increase in urinary loss.

Three principal adaptive mechanisms may be brought into play to defend the plasma albumin pool when this steady state is disturbed by the

development of albuminuria. The extravascular albumin pool may be mobilized into the intravascular space, the rate of albumin synthesis may be increased, or albumin catabolic rate may be decreased. Of these three adaptive mechanisms, only the last two are capable of reestablishing a steady state such that albumin production is again equal to the sum of external albumin loss plus catabolism.

ALBUMIN CATABOLISM

The bulk of albumin catabolism occurs in a compartment in rapid equilibrium with the vascular compartment and not in any predominant organ (109–112). Fibroblasts are one cell type that has been identified as contributing to albumin catabolism (113). In the absence of renal disease, approximately 10% to 20% of albumin catabolism takes place in the kidney (114) and this represents the amount of albumin filtered by the normal glomerulus (115,116). When glomerular filtration of albumin is increased, more albumin is presented to the proximal tubular cells and it is possible for the rate of renal albumin catabolism to be increased. The proximal tubule is capable of reabsorbing and recycling filtered albumin (43,117,118) under the control of a variety of receptor proteins, and increased podocyte uptake of albumin may be increasingly driven in part by increased FFAs bound to the albumin as a secondary consequence of disordered lipid metabolism (119). However, when glomerular permselectivity is greatly altered, most of the increased albumin filtered by the abnormal glomerulus is lost in the urine and not catabolized by the renal tubular epithelium. Therefore, urinary albumin excretion is a gross underestimate of the total albumin lost from the total body albumin pool in the nephrotic syndrome.

ALBUMIN SYNTHESIS

Albumin synthesis is predominantly regulated by the availability of adequate dietary protein (120–123) and is suppressed during inflammation (124,125). and metabolic acidosis (126). The rate of albumin synthesis is increased under conditions when plasma colloid osmotic pressure (*p)* is reduced, such as during nephrotic syndrome but appears to have an upper limit of approximately 25 to 30 g/day (95). Albumin synthesis is increased as a consequence of increased transcription of the cognate gene (127,128), regulated by the transcription factors early growth response factor-1 (EGRF-1) and hepatocyte nuclear factor-4 (HNF-4). Synthesis of albumin

in the nephrotic syndrome is positively associated both with that of several negative acute-phase proteins (apo A-I, transferrin) and positive acute-phase proteins (fibrinogen, α_2 macroglobulin) in part because of control by similar trans-acting factor possibly providing a linkage between dysregulation of a number of proteins characterized by increased synthetic rates in the nephrotic syndrome (129,130). As this regulation does not appear to be linked to the molecular weight of the proteins, some are lost in the urine as is albumin, and have a decreased plasma concentration as a consequence, and some are not, leading to an increase in their concentration in the plasma of nephrotic subjects (103).

Conditions that cause an increase in plasma *p* reduce the rate of albumin synthesis in vivo (131–133). Although albumin synthesis increases in direct proportion to albuminuria in both nephrotic patients and animals, the response fails to maintain albumin pools or plasma concentration in or near the normal range (95,134). There is no clear relationship between plasma albumin concentration and albumin synthetic rate in nephrotic patients (95) or animals (134,135). The reason for this is that serum albumin concentration primarily reflects renal albumin clearance because albumin synthetic rate is maximized in response to urinary albumin losses, constrained by dietary protein and other factors, such as inflammation. Albumin concentrations decline because daily urinary losses are of a magnitude similar to that of total albumin turnover rate.

Effect of Dietary Protein on Albumin Synthesis

The rate of albumin synthesis responds rapidly to acute changes in diet. When severely malnourished animals or people are fed, the rate of albumin synthesis increases promptly, although total body protein stores still are severely depleted (136,137). The most important nutritional constituent is dietary protein. The maintenance of a normal plasma albumin concentration and a normal rate of albumin synthesis depends on both total protein availability in the diet and the relative proportion of protein to nonprotein calories. Diets that provide adequate calories but are poor in protein have a more deleterious effect on albumin synthesis and on albumin stores than do diets that contain the same amount of protein but are deficient in calories (138,139). A balanced diet that is inadequate in both protein and calories does not cause hypoalbuminemia. A diet containing adequate calories but insufficient protein results in reduced albumin synthesis, albumin concentration, and total body albumin mass

(140) producing kwashiorkor. One would predict that an ideal diet for patients with the nephrotic syndrome, a disorder that bears much similarity to protein malnutrition, would contain adequate calories, but above all an adequate or preferably high protein content. Diets containing large excesses of protein, 3 to 4 g/kg body weight, have been prescribed in the past (141), although no data are available demonstrating the effectiveness of these diets in restoring protein pools. Increased dietary protein intake in fact fails to increase either albumin concentration or body albumin pools in patients with the nephrotic syndrome (142) (Fig. 14-9) or animals with experimentally induced nephrotic syndrome (123,135,143,144). Instead, much of the ingested protein is catabolized rather than used for net protein synthesis, and dietary protein augmentation also increases renal albumin clearance, causing any increased albumin that is synthesized to be lost in the urine. Furthermore, the increased albumin synthesis that results from dietary protein augmentation is accompanied by an increased rate of high-molecular-weight proteins, fibrinogen and of α_2 macroglobulin (145,146) that may play a role in the coagulopathy associated with the nephrotic syndrome, as will be discussed subsequently. In addition, dietary protein exerts an effect on the kidney, causing a reversible increase in glomerular permeability to large macromolecules (147), so most of the additional albumin synthesized is lost in the urine. Figure 14-10 shows the effect of diets containing either 2 or 0.6 g/kg of protein on the renal clearance of neutral dextrans when fed to nephrotic patients. It can be seen clearly that patients clear high-molecular-weight dextrans more easily when fed a high-protein diet. Thus, a change in dietary protein may alter the permselectivity characteristics of the glomerular filtration barrier in these patients increasing the renal clearance of albumin so that the net effect may be one of decreasing albumin stores (95,134).

Virtually every study of the effect of altered dietary protein intake on nephrotic syndrome noted that urinary albumin or protein excretion varied with dietary protein intake (146–149). Dietary protein has clearly been shown to increase glomerular hyperfiltration (142) and is associated with greater loss of residual renal function (148). Very low-protein diets have not been shown to reduce the risk of progression of renal disease compared to less severe restriction (150). It should be noted that the effect of dietary protein on albumin homeostasis in nephrotic patients has compared a usual protein intake (approximately 1.2 g/kg) to a modest level of protein restriction (0.8 g/kg), a value that more closely approaches the control group in studies of the effect of protein restriction on loss of renal function (151). Continued maintenance of a high-protein diet may have the

consequence of causing permanent rather than transient changes in the kidney and accelerate the progression of renal diseases (148,149,152). Increased filtration and tubular metabolism of plasma proteins may increase the injurious effect of high protein intake (153).

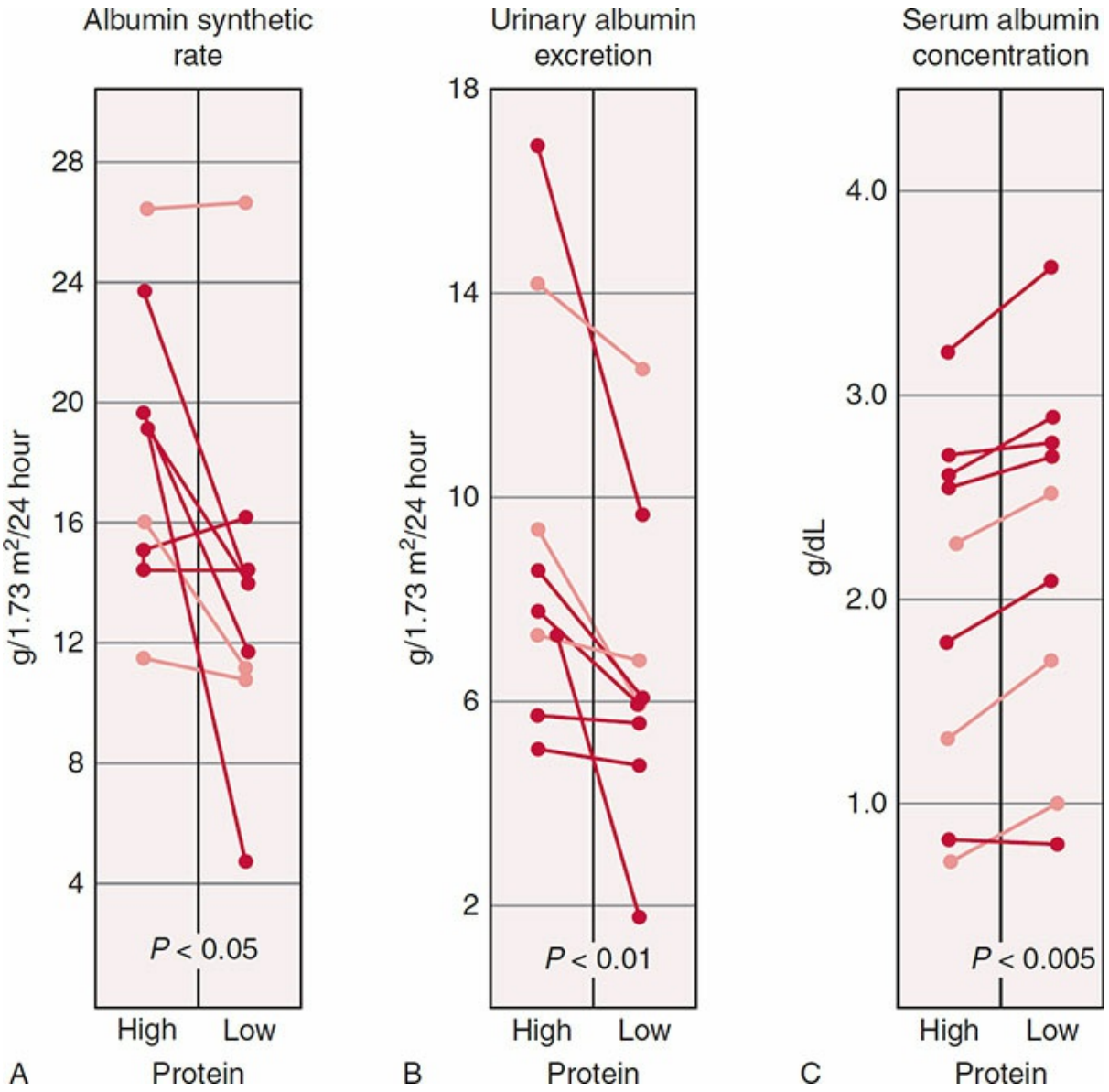

Figure 14–9 Changes in albumin synthetic rate **(first panel)**, in urinary albumin excretion **(second panel)**, and in the serum albumin concentration **(third panel)** that occur with isocaloric reduction in dietary protein intake in patients with the nephrotic syndrome of various etiologies. Closed circles represent the mean values for the group. (From Kaysen GA, Kirkpatrick WG, Couser WG. Albumin homeostasis in the nephrotic rat: nutritional considerations. *Am J Physiol*. 1984;247:F192–F202.)

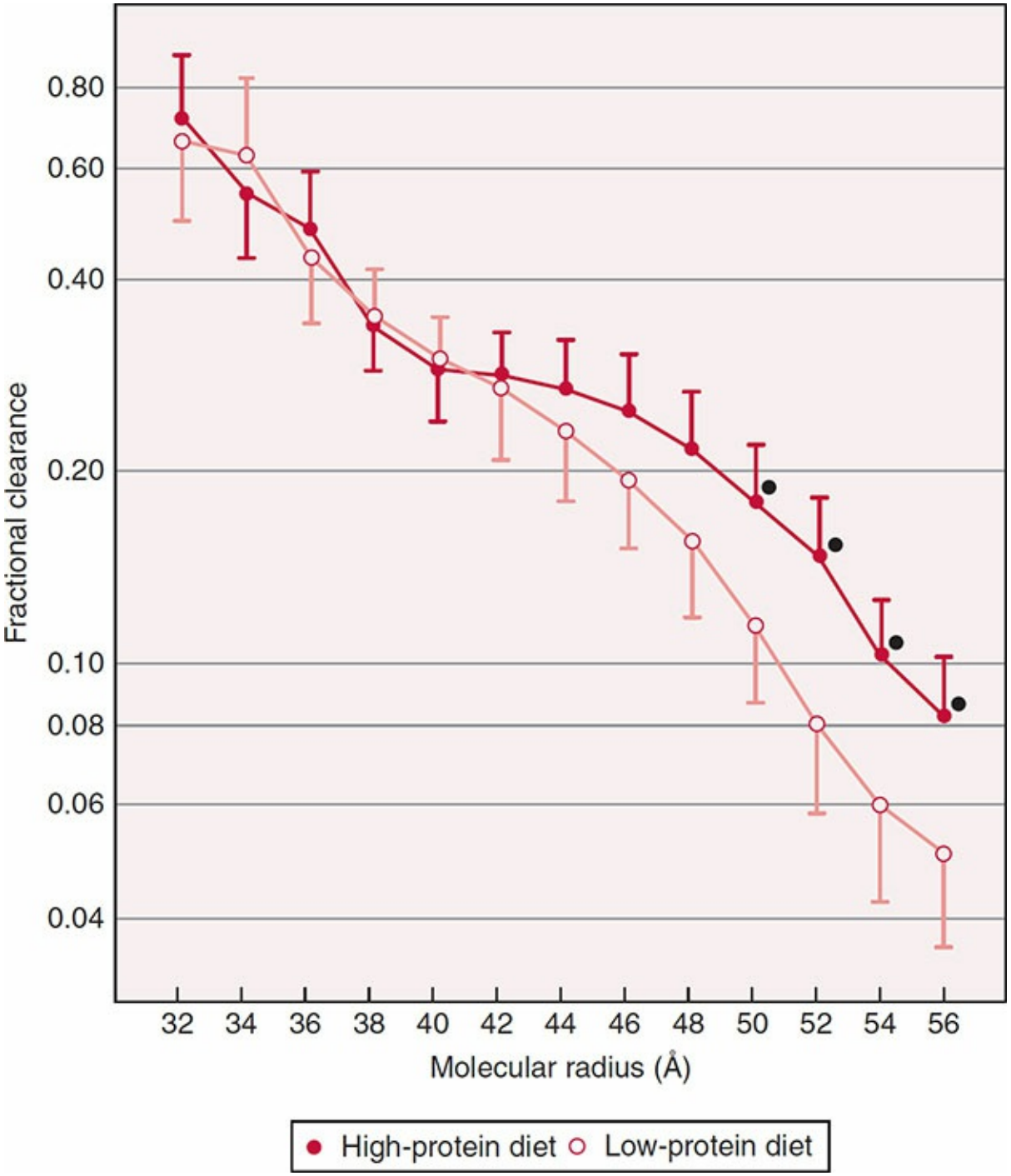

Figure 14–10 Effect of a high-protein diet (*solid symbols*) or low-protein diet (*open symbols*) on the fractional renal clearance of neutral dextrans in nephrotic patients. (Reprinted from Rosenberg ME, Swanson JE, Thomas BL, et al. Glomerular and hormonal responses to dietary protein intake in human renal disease. *Am J Physiol Renal Fluid Electrolyte Physiol*. 1987;253(22):F1083–F1090, with permission.)

The rate of albumin synthesis increases in parallel with urinary protein loss (103) and dietary protein intake (146), although at steady state it is difficult to sort out which process is primary. At steady state, urinary albumin losses can only equal albumin synthesis rate minus albumin

catabolic rate so that increasing urinary albumin losses require increased albumin synthetic rate. In the nephrotic syndrome, the rate of albumin synthesis correlates with that of other proteins, many of which have pathologic consequences. One of these is fibrinogen (103) (Fig. 14-11); another is the atherogenic lipoprotein Lp(a) (Fig. 14-12) (132). Of interest, restriction of dietary protein reduces fibrinogen synthesis in nephrotic patients. Fibrinogen levels in nephrotic patients are directly proportional to the rate of synthesis of this protein (103) and because fibrinogen is a powerful cardiovascular risk factor, reducing its rate of synthesis by avoiding a high-protein intake has the potential of reducing this risk factor.

Although augmentation of dietary protein also causes an increase in the rate of albumin synthesis in both animals and patients with the nephrotic syndrome, neither protein concentration nor albumin concentration increases as a consequence (134,135). The reason lies in the fact that these three processes offset one another, so albumin concentration actually may tend to decrease during consumption of a high-protein diet. If the increase in urinary albumin excretion that follows dietary protein augmentation is prevented by administration of an ACEI, a high-protein diet will cause an increase in albumin concentration in nephrotic rats (147), and increases nitrogen balance in nephrotic patients (154).

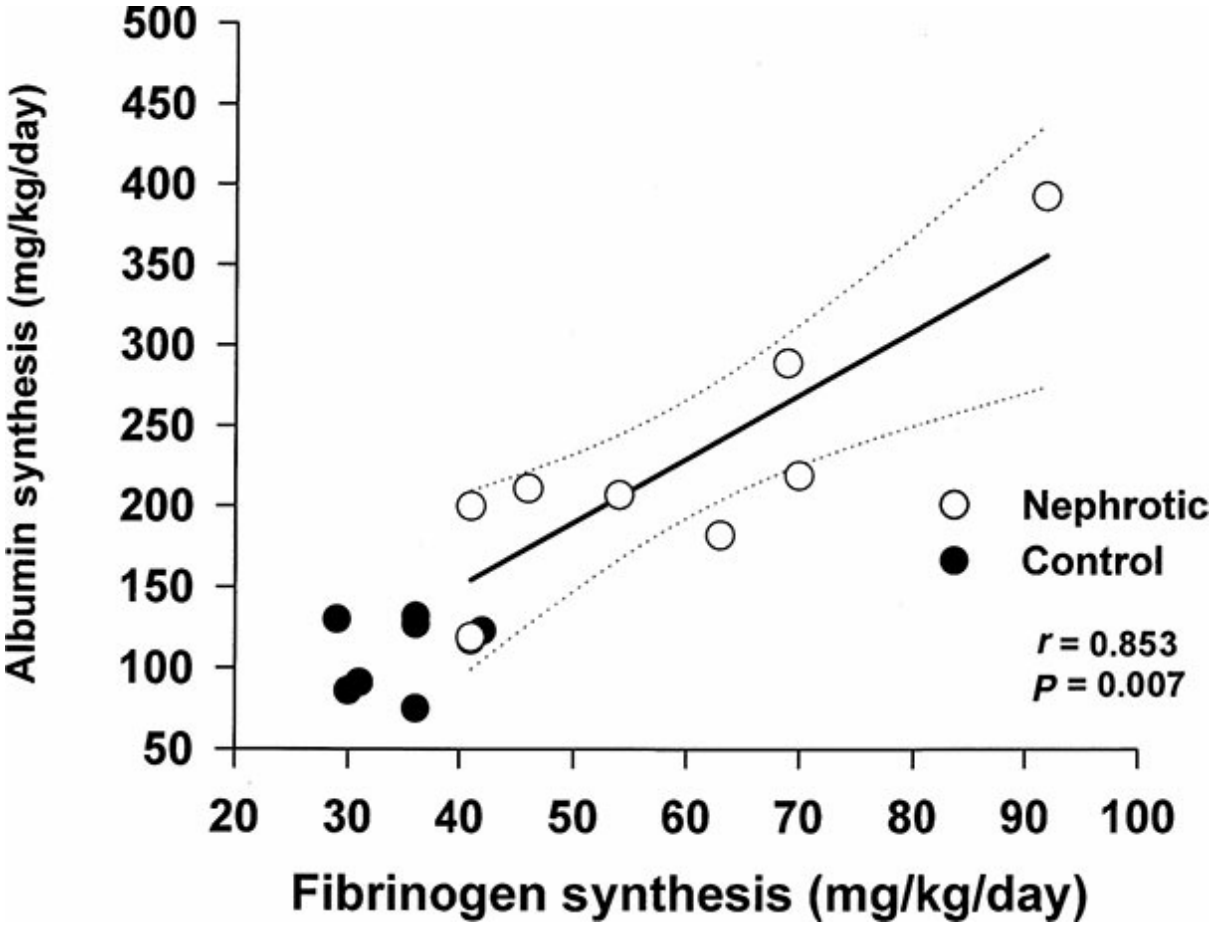

Figure 14–11 Relationship between albumin and fibrinogen synthesis in nephrotic patients (*open symbols*) and control subjects (*closed symbols*) measured as the incorporation of 13C valine. (Reprinted from de Sain-van der Velden MG, Kaysen GA, de Meer K, et al. Proportionate increase of fibrinogen and albumin synthesis in nephrotic patients: measurements with stable isotopes. *Kidney Int.* 1998;53 (1):181–188 with permission from Elsevier.)

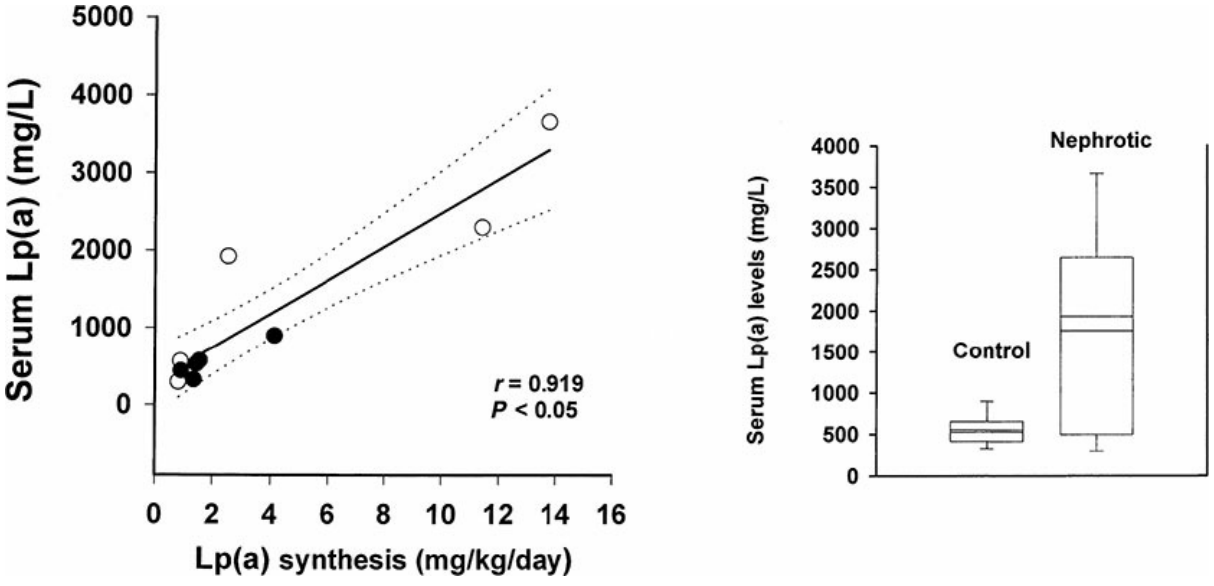

Figure 14–12 Comparison of Lp(a) levels in nephrotic patients in comparison with

control subjects **(right panel)** and relationship between plasma Lp(a) levels and Lp(a) synthetic rate in nephrotic patients **(left panel)**. (Data from de Sain-van der Velden MG, Jan Riejngoud D, Kaysen GA, et al. Evidence for increased synthesis of Lipoprotein (a) in the nephrotic syndrome. *J Am Soc Nephrol*. 1998;9: 1474–1481.)

The therapeutic approach to maximizing albumin concentration in nephrotic patients primarily should be aimed at reducing urinary albumin excretion, not increasing the rate of albumin synthesis, especially as this may be accompanied by increased synthesis and accumulation of proteins that may have a deleterious effect, especially on blood coagulation.

METABOLISM OF NONALBUMIN SERUM PROTEIN IN NEPHROTIC SYNDROME

Plasma protein composition is greatly altered in the nephrotic syndrome (155). Albumin and proteins of similar size are lost in the urine, and their concentration in plasma is decreased (156). In contrast, the plasma concentration of several proteins of high molecular weight is increased (157). Urinary protein loss is accompanied by increased synthesis of several proteins secreted by the liver (158). For the most part, the compensatory response to urinary protein loss, if indeed the response can be viewed as compensatory, is an increased synthesis of specific proteins secreted by the liver. The response is confined almost, if not entirely, to the liver. Indeed total body protein synthesis and turnover is unaffected by nephrotic range proteinuria (159). For example, the synthesis of both apo A-I and transferrin is increased in the liver in the nephrotic syndrome (160), whereas there is no change in synthesis of apo A-I by the gut (161), the other organ that secretes apo A-I, and there is no change in transferrin gene expression by extrahepatic tissues (162). Regulation of apo A-I is based at least in part by upregulation of the early growth response factor 1 (EGR-1) (129). Upregulation of synthesis of proteins regulated both by this transcription factor and HNF-4 (127) appears to be coordinately upregulated both in the nephrotic syndrome and in analbuminemic rats suggesting that a cohort of proteins are regulated in response to reduced oncotic pressure.

Lipids in the form of oval fat bodies may appear in the urine during the nephrotic syndrome when proteinuria is extensive. At least part of this phenomena may be explained by failed tubular uptake of filtered proteins, specifically high-density lipoprotein (HDL), that are not adequately catabolized by the proximal nephron as a consequence of injury engendered by protein overload. Approximately 25% of HDL catabolism

occurs in the kidney following glomerular filtration (163). As glomerular proteinuria increases, so does the excretion of smaller proteins normally taken up and catabolized, most likely as a consequence of secondary injury to the proximal tubule as a result of continued massive proteinuria.

Proteins Involved in Hematopoiesis

Proteinuria can have more than one pathway to interfere with hematopoiesis, although the relationship between proteinuria and anemia is variable (102,164,165). Urinary protein losses may lead to decreases in the iron transport proteins (transferrin) and hematopoietic stimulatory proteins (erythropoietin) and also may result in specific injury to the renal tubules that alter production of erythropoietin. Transferrin has a molecular weight of 79.5 kDa and is the principal iron carrier protein in plasma. Each mole of this protein lost in the urine carries potentially 2 moles of iron. Microcytic hypochromic anemia has been described in the nephrotic syndrome, although this is uncommon (166–168) and has been attributed to iron loss. No clear relationship between urinary total protein losses and anemia has been demonstrated (164), suggesting that the relationship between anemia and proteinuria is more complex than simply a linear relationship between blood hemoglobin concentration and a protein lost in the urine.

Transferrin synthesis is increased in the nephrotic syndrome (169) and this response is confined to the liver, as in the case of iron deficiency (170). Unlike iron deficiency, however, augmentation in transferrin gene expression cannot be suppressed by administration of even large amounts of iron parenterally, and transferrin synthesis also is increased in hereditary analbuminemia (171), a condition not associated with urinary iron loss. In patients with the nephrotic syndrome, transferrin synthesis increases proportionally to that of albumin synthesis, suggesting regulation of synthesis of these two proteins is linked in this disorder, similar to that of other proteins, such as fibrinogen. These findings suggest that augmentation in transferrin synthesis is not proof of iron deficiency but more likely represents part of a systemic response to decreased oncotic pressure. Iron deposited in the renal tubules from reabsorbed transferrin also may play a role in the putative nephrotoxic effects of proteinuria (172). In addition, transferrin, but not albumin, may alter expression of the genes encoding aquaporin 1 and 3 affecting renal handing of water (173).

Erythropoietin is synthesized by the kidney and regulates red blood

cell levels. This protein also is lost in the urine in both nephrotic patients (100) and rats (174) but synthesis of erythropoietin, like that of other non–liver-derived proteins, is not increased in response, and plasma levels are decreased, as is the hematocrit in nephrotic rats. Additionally proteinuria per se may injure renal production of erythropoietin (175) adding to deficiencies resulting from urinary loss. Erythropoietin deficiency could potentially play a role in the development of anemia in some nephrotic patients, although this has not been established (174). Although urinary iron excretion also increases in the nephrotic syndrome as a consequence of urinary transferrin loss (169), the loss of erythropoietin may contribute to the development of anemia. No controlled studies have been performed to determine whether administration of either iron or erythropoietin corrects anemia in nephrotic patients so as to test the hypothesis that erythropoietin or iron deficiency is responsible for anemia in some nephrotic patients.

Vitamin B_{12} is carried by the protein transcobalamin, having a molecular weight of 43 kDa. This protein has been found to be lost in the urine of subjects with a form of congenital nephrotic syndrome to an extent where it is undetectable in serum, resulting in profound vitamin B_{12} deficiency, contributing to depressed erythropoiesis through another non–iron-mediated mechanism (102).

Immunoglobulin Metabolism

Hypogammaglobulinemia has long been recognized as a serious manifestation of the nephrotic syndrome (176–178) and is an important factor in the reduced defenses against bacterial infections (179) in nephrotic patients. In congenital nephrotic syndrome, immunoglobulins may be absent entirely (178). In addition to albumin, IgG is lost in the urine when glomerular permselectivity is severely altered (33). The urinary loss of this protein undoubtedly plays a significant role in causing hypogammaglobulinemia in the nephrotic syndrome. As glomerular permselectivity is progressively lost, the renal clearance of IgG approaches that of albumin, despite the much larger effective molecular radius of IgG. Like albumin, the fractional catabolic rate of IgG varies directly with concentration in humans and rodents, increasing from 2% during severe hypogammaglobulinemia to as high as 18% when IgG concentration is high (180). As in the case of albumin metabolism, the increased fractional

rate of IgG catabolism is inappropriate because of the presence of hypogammaglobulinemia in nephrotic patients (181). This phenomenon most likely reflects increased renal catabolism of IgG despite a decrease in IgG catabolism elsewhere in the body.

Although IgG production may be increased in vivo in patients with the nephrotic syndrome (177,182), the rate of IgG production is depressed (183,184) in lymphocytes isolated from patients with the nephrotic syndrome of various etiologies when they are exposed to mitogens in culture. This apparent contradiction has not yet been resolved, but it must be remembered that the nephrotic syndrome may be the consequence of immunologically mediated disease in some situations, and changes in Ig may reflect the underlying disease and not the physiologic response to urinary protein loss or changed plasma protein composition.

It has been proposed that the urinary losses of IgG alone cannot be an adequate explanation for the low blood levels because the various subclasses of IgG are depressed asymmetrically (185), but it is more likely that IgG levels fall because, unlike the case of liver-derived proteins of the same size class, there is no compensatory increase in the IgG synthetic rate. When the nephrotic syndrome is induced in experimental animals, there is no increase in IgG synthesis, and both plasma levels and total body pools are dramatically reduced (186). Ultimately, at the final steady state, very little IgG is found in the urine because plasma levels are so low.

Of the Igs, IgG is most severely depleted in the nephrotic syndrome (187), most likely because it is smallest and its renal clearance greatest. IgA levels also are reduced but less so. In contrast to IgG, IgM levels are increased (188). Although it has been speculated that increased IgM levels might play a role in causing some forms of the nephrotic syndrome, such as minimal change nephrotic syndrome, this is unlikely because the increase in IgM concentration has been reported almost universally. The increase in concentration of this very large, essentially unfilterable protein is similar to the response of many liver-derived proteins (189–191), the metabolism of which is reviewed in the following sections.

Defects in Hormone-Binding Proteins

Thyroid-binding globulin is found in the urine of nephrotic patients (192–195), but the concentration of this protein is reduced only in patients with extremely high urinary protein output. Nephrotic patients are euthyroid, as serum thyrotropin levels are not increased, and thyroid

function tests, as assessed by radioactive iodine uptake, are normal. Similarly, although steroid-binding proteins (193) (corticosteroid-binding globulin) are reduced, there is no evidence that this leads to clinically significant reductions in free corticosteroid levels.

Vitamin D–Binding Protein and Hypocalcemia in the Nephrotic Syndrome

Hypocalcemia has been long recognized in nephrotic patients (196,197) but it was realized only recently that ionized calcium as well as total calcium are reduced. Vitamin D–binding protein is lost in the urine in the nephrotic syndrome governed at least in part by alteration is permselectivity (198). The urinary loss of vitamin D–binding protein (65 kDa) in the nephrotic syndrome (196) may lead to major derangements in calcium metabolism. Therefore, hypocalcemia does not result entirely from a reduction in the fraction of calcium bound to albumin. Vitamin D levels are reduced (199,200), and the decrease in vitamin D concentration correlates with urinary albumin excretion and decreased bone density (201). Albumin concentration and vitamin D concentration correlate closely. Vitamin D–binding protein also is identifiable in the urine of nephrotic patients and vitamin D levels normalize when proteinuria resolves (202,203). It is not known whether synthesis of vitamin D–binding protein is altered in response to its urinary loss or is modulated by dietary protein intake. Labeled vitamin D appears rapidly in the urine of nephrotic subjects (204). Hypovitaminosis D is not the result of loss of renal mass, because serum vitamin D levels are suppressed in nephrotic patients with normal renal function (199,200). Although it is possible that proteinuria might in some way inhibit vitamin D_1-hydroxylase, an enzyme located in the renal proximal tubule, such an explanation seems unwarranted. Hypovitaminosis D of the nephrotic syndrome may cause rickets (osteomalacia), especially in children (204,205). Nephrotic patients malabsorb calcium, a defect that can be corrected with exogenously administered vitamin D (206). Moreover, it has been recognized that vitamin D has pleiotrophic effects beyond its importance in bone mineralization (207). Vitamin D reduces cell proliferation and inflammation and has antineoplastic properties. Vitamin D deficiency has been associated with elevated blood pressure, reduced vascular compliance, impaired wound healing, and increased cancer rates and

mortality. Thus, there may be additional morbidity in nephrotic patients related to vitamin D deficiency beyond impaired bone mineralization. However, unlike many of the other manifestations of the nephrotic syndrome, hypovitaminosis D can be managed with replacement therapy.

METABOLISM OF HIGH MOLECULAR WEIGHT SERUM PROTEINS IN THE NEPHROTIC SYNDROME

The concentration of several proteins that are not lost in the urine or are lost in only limited amounts is increased in plasma because of their increased hepatic synthesis (145,158). The hepatic response to urinary protein loss is an increase in synthesis rate both of proteins lost in the urine and of those not lost. When examined, the mechanism is one of increased gene transcription. The increased plasma concentration of several of these proteins (β, α_1, and α_2 macroglobulins) is not associated with adverse clinical outcomes. In contrast, the increased concentration of lipids or fibrinogen may pose atherogenic risk, as will be discussed subsequently, and the increased concentration of several large proteins involved in hemostasis contributes to the thrombotic tendency that complicates the nephrotic syndrome.

Thrombosis

The nephrotic syndrome is complicated by venous thrombosis (208,209). Renal vein thrombosis results from, rather than causes, the nephrotic syndrome (210,211). Both pulmonary emboli and renal vein thrombosis, while frequently asymptomatic, are quite common and occur in as many as 30% to 35% of adult patients and a potentially higher number of children (212) with the nephrotic syndrome (213,214). Risk factors, other than cause of the nephrotic syndrome, that predicted thrombosis were high factor VIII and decreased antithrombin III level (213). The significant increase in thromboembolic disorders is caused in part by the urinary loss of several proteins that are inhibitors of blood coagulation, specifically antithrombin III (190), proteins S and C (215,216), protein Z, a vitamin K–dependent protein (156), and the increased plasma concentration of several high-molecular-weight proteins, including the binding proteins for proteins C and S. Of all of the changes in plasma protein composition associated with increased thrombotic tendencies, hypoalbuminemia (217,218) and

disease severity (219) have the highest association.

The plasma concentration and the hepatic synthesis of fibrinogen (340 kDa) are both increased in the nephrotic syndrome (103). Although total proteins C and S may be elevated (216) in the nephrotic syndrome, these measurements reflect the total concentration of the proteins. An increased total concentration results from an increment in the plasma concentration of their high-molecular-weight carrier protein, C4b (220). The plasma concentration of the biologically active free form of these intermediate-molecular-weight inhibitors of blood coagulation is decreased as a result of both their increased urinary loss and their increased binding in inactive form to C4b. The combination of the increased concentration of high-molecular-weight procoagulants and decreased concentration of intermediate-molecular-weight anticoagulants produces the clotting diathesis that complicates the nephrotic syndrome.

Hyperlipidemia

The characteristic disorder in blood lipid composition in nephrotic patients is an increase in the low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) (220), and/or intermediate-density lipoprotein (IDL) fractions but no change or a decrease in HDL (221) despite an increase in apo A-I synthetic rate (222), resulting in an increase in the LDL/HDL cholesterol ratio. Lipoprotein particles rich in phospholipid and esterified and nonesterified cholesterol resembling VLDL remnants (IDL) and chylomicron (CM) remnants accumulate. Apos B and C-III are increased in the serum of nephrotic patients, but the concentrations of apo A-I, A-II, and C-II remain unchanged. HDL subtypes found in plasma of nephrotic patients also are abnormally distributed (133). HDL_3 is modestly elevated, whereas HDL_2 is markedly reduced. Because it is primarily the latter subclass of HDL that is protective against atherosclerosis, the combination of reduced HDL_2 in conjunction with increased LDL, VLDL, and IDL cholesterol potentially poses significant risk for cardiovascular disease.

Lp(a) is a prominent risk factor in atherogenesis and coagulopathies (223,224). Generally, the quantity of this lipoprotein in plasma is genetically determined (225). Lp(a) consists of a molecule of LDL to which one molecule of apo(a) is covalently attached to apo B 100. The size of the apo(a) molecule in Lp(a) is genetically determined and distributed in the population in a non-normal fashion (226). Individuals having the

largest apo(a) subtypes are most common and have the lowest plasma concentration of Lp(a). Lp(a) levels are increased in patients with a variety of renal diseases, including the nephrotic syndrome (227). Unlike inherited increases in plasma Lp(a) levels, these increases in Lp(a) are acquired and are not associated with the increased size of apo(a) (228).

Decreased Lipoprotein Catabolism

Hyperlipidemia in the nephrotic syndrome is a consequence of two separate and distinct processes—increased synthesis as discussed above, and decreased clearance. This is a consequence of an inability to efficiently clear triglyceride (TG)-rich lipoproteins from plasma. These lipoproteins consist primarily of VLDL, CMs, and remnant particles. Although some investigators have shown VLDL synthesis to be increased, this is not the primary cause for its increased concentration. VLDL apo B 100 synthesis can be increased in patients, although the levels of the protein remain quite normal. There is no difference between the absolute synthesis rate of apo B 100 in VLDL in nephrotic subjects and controls, and when the subjects with the nephrotic syndrome are divided between normal levels of TGs (<2.5 mM) and elevated levels, the absolute synthesis rate of apo B 100 in VLDL actually tends to be increased in patients with lower rather than greater TG levels. By contrast, in Figure 14-13, one can see that although the absolute rate of apo B 100 has no relationship to VLDL TG levels, the fractional rate of apo B 100 synthesis (which is equal to the fractional catabolic rate of VLDL) indeed is inversely related to VLDL TG levels. Thus, it is the catabolic rate and not the synthetic rate that controls VLDL levels in nephrotic patients.

One is a decreased quantity of LPL bound to the endothelial surface (229). This is a result of reduced serum albumin concentration and is found in the absence of albuminuria in the condition of hereditary analbuminemia (230). As an isolated defect, the reduction in lipase leads to, at the most, a small increase in blood lipid levels (Fig. 14-14). The second process involves an inactivation of LPL by increased levels of angiopoietin-like protein 4 (Angptl4) (37). Although LPL mRNA levels are normal in peripheral tissue consistent with a normal rate of synthesis of this enzyme, the circulating activity of LPL is severely diminished. Defective clearance of TG-rich lipoproteins is linked directly to the appearance of proteinuria (231). The albumin that is lost trans-glomerularly contains only small amounts of bound FFAs leaving fatty

acid saturated isoforms of albumin enriched in the plasma of nephrotic subjects. The fatty acid is delivered to muscle, adipose tissue, and cardiac tissue, and stimulates the release of the LPL inhibitor Angptl4 linking the defect in glomerular permselectivity, the urinary loss of albumin, and the defect in clearance of TG-rich lipoproteins. Of interest, the induction of proteinuria in rats with hereditary analbuminemia, the Nagase analbuminemic rat (NAR) generates an identical level of TG-rich lipoproteins as does the initiation of proteinuria in an otherwise normal rat, suggesting that proteins other than albumin, or in addition to albumin, contribute to the cyclic relationship between proteinuria, increased synthesis of Angptl4, and feedback reduction in the sieving defect mediated by the isoform of Angptl4 secreted by muscle, fat, liver, and myocardium (37). Angptl4 levels increase in plasma both in patients with the nephrotic syndrome because of a variety of causes and in rats with the experimentally induced nephrotic syndrome, despite urinary losses of this protein.

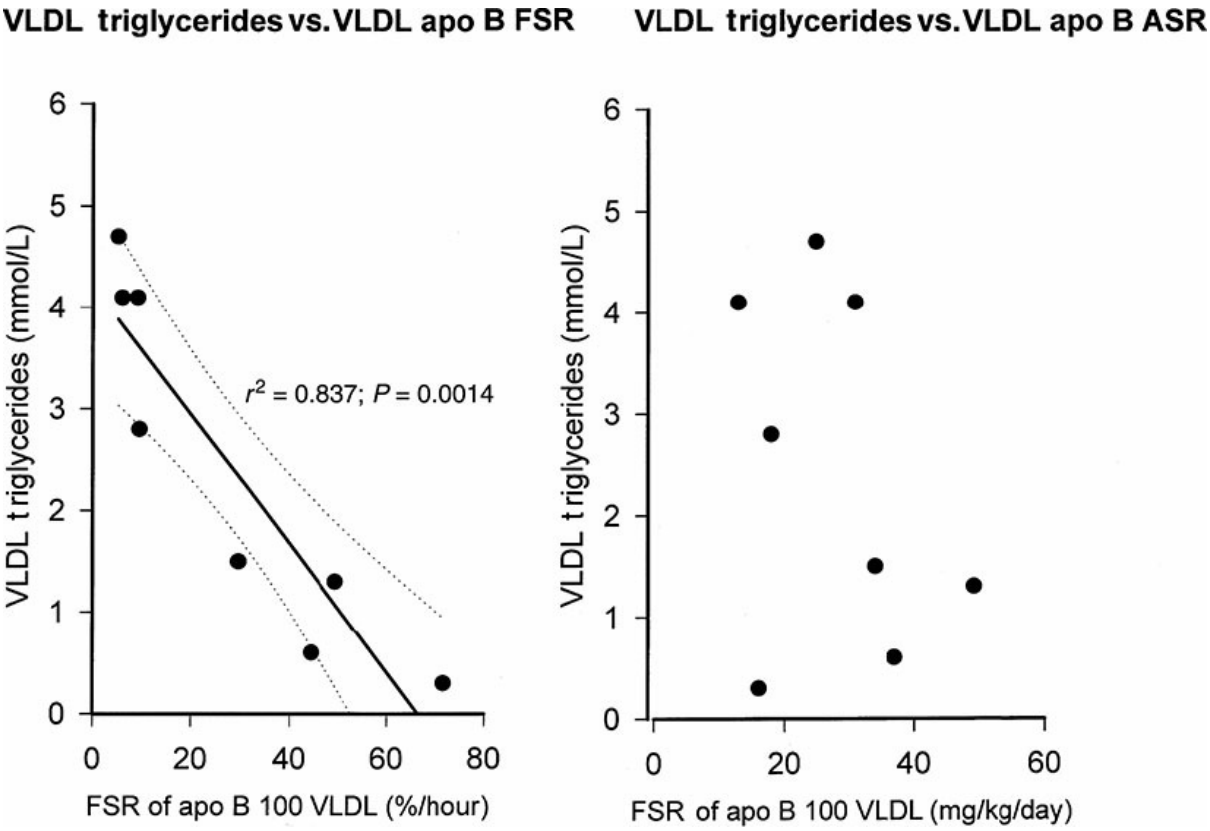

Figure 14–13 Relationship between plasma apo B 100 very-low-density lipoprotein (VLDL) (mg/L) and the fractional catabolic rate (FCR) of apo B 100 (%/day) in nephrotic patients (**left panel**). Ninety-five percent confidence limits of the whole group are shown on either side of the regression line; r^2 = 0.708, P = .0088 (n = 8). Relationship between plasma apo B 100 VLDL (mg/L) and the absolute synthetic rate

of apo B 100 VLDL (mg/kg/d) for nephrotic patients; r^2 = 0.25, P = .21 (n = 8) (**right panel**). (Reprinted from de Sain-van der Velden MG, Kaysen GA, Barrett HA, et al. Increased VLDL in nephrotic patients results from a decreased catabolism while increased LDL results from increased synthesis. *Kidney Int*. 1998;53(4):994–1001, with permission from Elsevier.)

Angptl4 participates in two separate feedback loops. First, its release from mesenchymal tissue upon exposure to FFA delivered by albumin results in suppression of LPL and decreases the presentation of FFA to these tissues (232), at the expense of increased levels of unhydrolyzed TGs carried in VLDL and IDL. Second, Angptl4 binds to glomerular endothelial $\alpha_v\beta_5$ integrin and reduces glomerular sieving of proteins, reducing proteinuria (37). Another protein that appears to be involved in increasing fatty acid presentation to these tissues in the nephrotic syndrome is α_1-antitrypsin (233,234). This protein also increases tissue presentation of FFA resulting in increased release of Angptl4.

Of potential concern, patients who inherit a gene for Angptl4 that has a loss-of-function mutation have a decreased risk of coronary disease (235,236) providing a potential link between increased expression of this protein in the nephrotic syndrome and adverse cardiovascular outcomes.

The third process involves an alteration in the structure of VLDL, most likely mediated by an interaction with HDL. VLDL incubated with HDL obtained from nephrotic rats is catabolized at a reduced rate in in vitro systems and binds less effectively to LPL (237). The abnormality in the HDL structure has been hypothesized to be a consequence of the urinary loss of lecithin cholesterol transfer protein, an enzyme necessary for HDL maturation (237). In turn, HDL is important for regulating the structure of other lipoproteins.

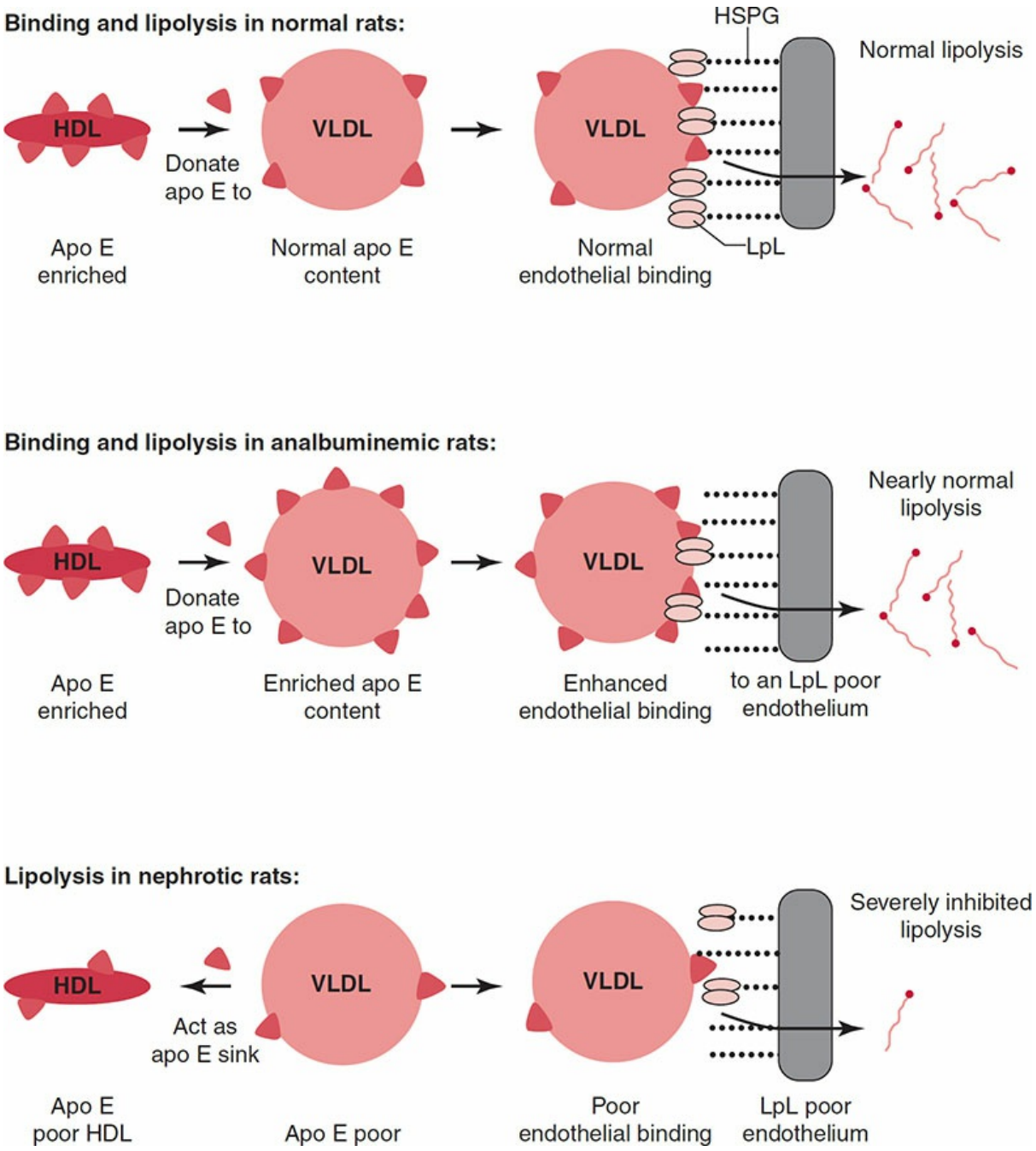

Figure 14–14 Model for defective binding and lipolysis of VLDL mediated by a shift of apo E to HDL in the nephrotic syndrome. Hypoalbuminemia leads to reduced amounts of lipoprotein lipase (LPL) bound to heparan sulfate on the endothelial surface. This alone leads to a slight decrease in lipoprotein catabolism and a mild increase in triglyceride levels. Proteinuria causes a decrease in apolipoprotein-bound apo E, a LPL ligand. This combined defect results in a marked reduction in lipoprotein catabolism. apo, apolipoprotein; HDL, high-density lipoprotein; HSPG, heparan sulfate proteoglycan; VLDL, very-low-density lipoprotein. (Reprinted from Shearer GC, Kaysen GA. Proteinuria and plasma compositional changes contribute to defective lipoprotein catabolism in the nephrotic syndrome by separate mechanisms. *Am J Kidney Dis*. 37(1, suppl 2):S119–S122, with permission from Elsevier.)

The abnormality in the lipoprotein structure that leads to its reduced catabolism is a consequence of urinary protein loss and not of hypoalbuminemia. Thus, the two separate components that comprise nephrotic syndrome, proteinuria, and hypoalbuminemia combine in these ways to produce this single defect in lipid levels (229,230). The decrease in HDL_2 and increase in HDL_3 are likely to play an important role in causing the defect in lipoprotein catabolism. HDL interacts with other lipoproteins in a number of important ways, but one consists of either acting as a source of apolipoproteins necessary for their catabolism or binding to receptors or ligands. Among these are apo E, a ligand that effects binding both to LPL as well as to several receptors; apo C-II, an activator of LPL; and apo C-III, an inhibitor of LPL. Both VLDL and HDL from nephrotic rats are depleted of apo E (237). It is the larger form of HDL, HDL_2, that effects this transfer most efficiently.

Table 14–4 Lipoprotein Content

	VLDL	HDL
Ratio	Apo E/Apo B (mol/mol)	Apo E/Apo A1
Control	5.77	0.34
HA	8.81	0.23
NS-Adriamycin	1.57	0.15

Apo lipoprotein composition of very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) isolated from normal control Sprague–Dawley rats, rats with hereditary analbuminemia (HA), and rats with the nephrotic syndrome induced by injection of adriamycin. Because each molecule of VLDL contains 1 mol of apo B, the ratio of apo E/apo B establishes the relative amount of apo E present per mole of VLDL.

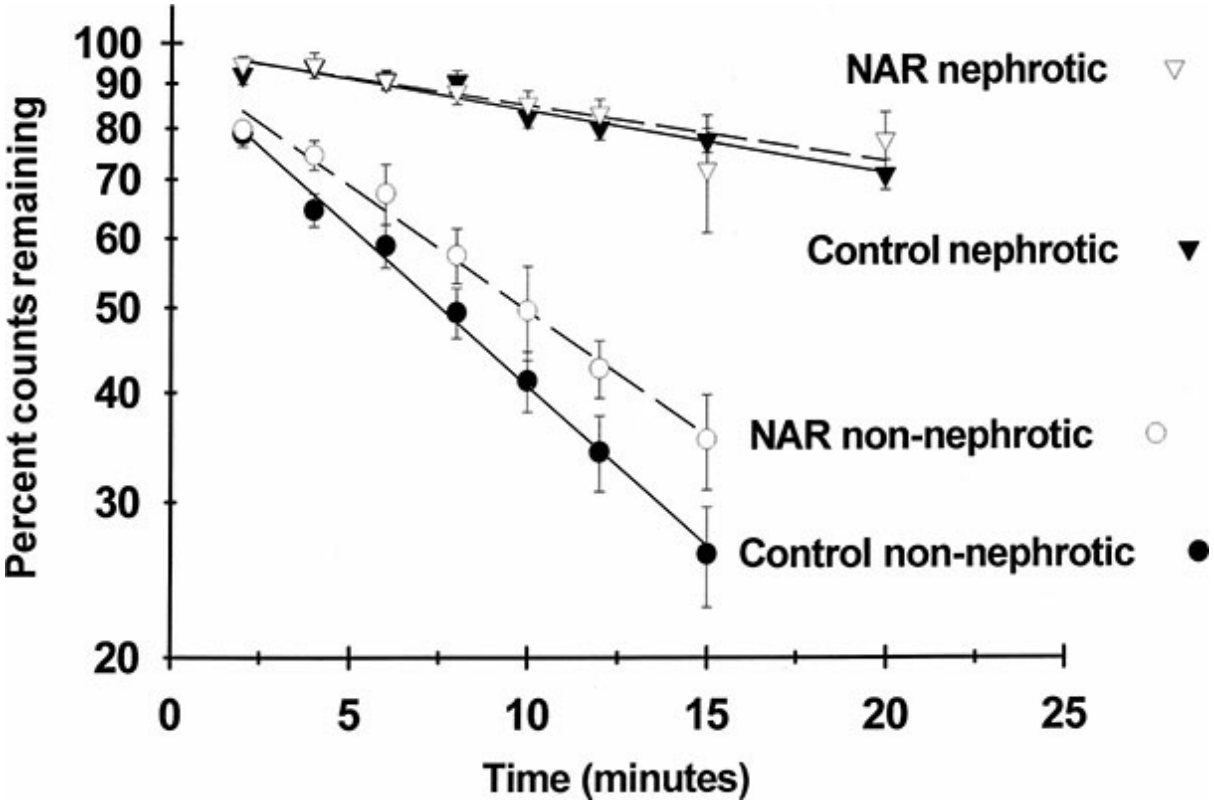

Figure 14–15 Chylomicron (CM) clearance in normal and nephrotic Sprague–Dawley and Nagase analbuminemic rats. CM clearance was measured by the disappearance of [^{3}H]-labeled CMs after intravenous injection. NAR, Nagase analbuminemic rat, represented by open symbols and broken lines. SD, Sprague–Dawley, represented by *solid symbols* and *lines*. Nephrotic rats are represented by *inverted triangles* and non-nephrotic rats by *circles*. The $t^{1/2}$ of each subgroup is indicated in the figure. Nephrotic NAR, $N = 7$; nephrotic SD, $N = 8$; normal NAR, $N = 6$; normal SD, $N = 6$. (Republished with permission of American Society for Clinical Investigation from Davies RW, Staprans I, Hutchison FN, et al. Proteinuria, not altered albumin metabolism, effects hyperlipidemia in the nephrotic rat. *J Clin Invest*. 1990;86:600–605; permission conveyed through Copyright Clearance Center, Inc.)

VLDL from nephrotic animals is catabolized by LPL at a reduced rate compared with control (238), but this defect can be repaired by incubation with HDL from normal animals but not following incubation with HDL from nephrotic animals. VLDL obtained from nephrotic rats also binds to LPL, the enzyme necessary for its catabolism, more poorly than does VLDL from control rats or from rats with hereditary analbuminemia (230). Both VLDL and HDL from nephrotic rats are depleted of apo E compared with control or analbuminemic animals (Table 14-4). Incubation of VLDL from nephrotic animals with HDL from normal animals repairs the binding defect, although incubation of VLDL from normal animals with HDL from nephrotic animals confers a binding defect. Thus, the defect in the VLDL structure and function is a consequence of its interaction with abnormal

HDL. Furthermore, injection of normal HDL into nephrotic rats partially repairs the defect in CM clearance in vivo (229). How is this related to other factors in the nephrotic syndrome?

Endothelial-bound LPL is greatly reduced both in analbuminemia and in the nephrotic syndrome, yet both VLDL and CM catabolism are not greatly impaired in analbuminemic rats. Rats with hereditary analbuminemia have mild hyperlipidemia and only minimal abnormalities in lipoprotein catabolism until they develop proteinuria (Fig. 14-15). Thus, urinary protein losses must contribute substantially to the disorder in lipid metabolism. A number of potential substances lost in the urine have been proposed to be responsible, but the most exciting observation was published by Vaziri et al. (237). They observed that the enzyme lecithin: cholesterol-acyltransferase (LCAT) was lost in the urine and depleted from plasma. This enzyme is necessary for normal HDL maturation and its uncompensated urinary loss could well explain the defective structure and function of HDL, which in turn would mediate the cascade of events leading to disordered catabolism of TG-rich lipoproteins.

One function of HDL is to shuttle apo C-II from remnant particles to nascent VLDL and CM (Fig. 14-16). Therefore, normal catabolism of both lipoproteins requires the presence of normally functioning HDL. HDL is derived from apos synthesized either in the liver or in the gut and cholesterol and phospholipids released by lipolysis of other lipoproteins. HDL initially appears as discoid nascent HDL, containing little or no cholesterol esters (239). Surface cholesterol is esterified by the enzyme LCAT (240). Phospholipids are hydrolyzed; the fatty acid, usually arachidonate, is combined to cholesterol to form cholesterol ester; and a mole of lysolecithin is liberated. As in the case of fatty acid transport, albumin serves to bind liberated lysolecithin and accelerates activity of LCAT, thus facilitating the maturation of HDL (241). The hydrophobic cholesterol esters formed by the LCAT reaction sink into the core of nascent (discoid) HDL and form a spheroid HDL_3 particle with a molecular weight of about 200 kDa. By further action of LCAT, HDL_3 is converted into the 400-kDa HDL_2 particle, a form of HDL more capable of transporting apo C-II. Without recycling of apo C-II by HDL_2, the action of LPL on CM and VLDL is greatly reduced. The nephrotic syndrome in humans is characterized by reduced HDL_2.

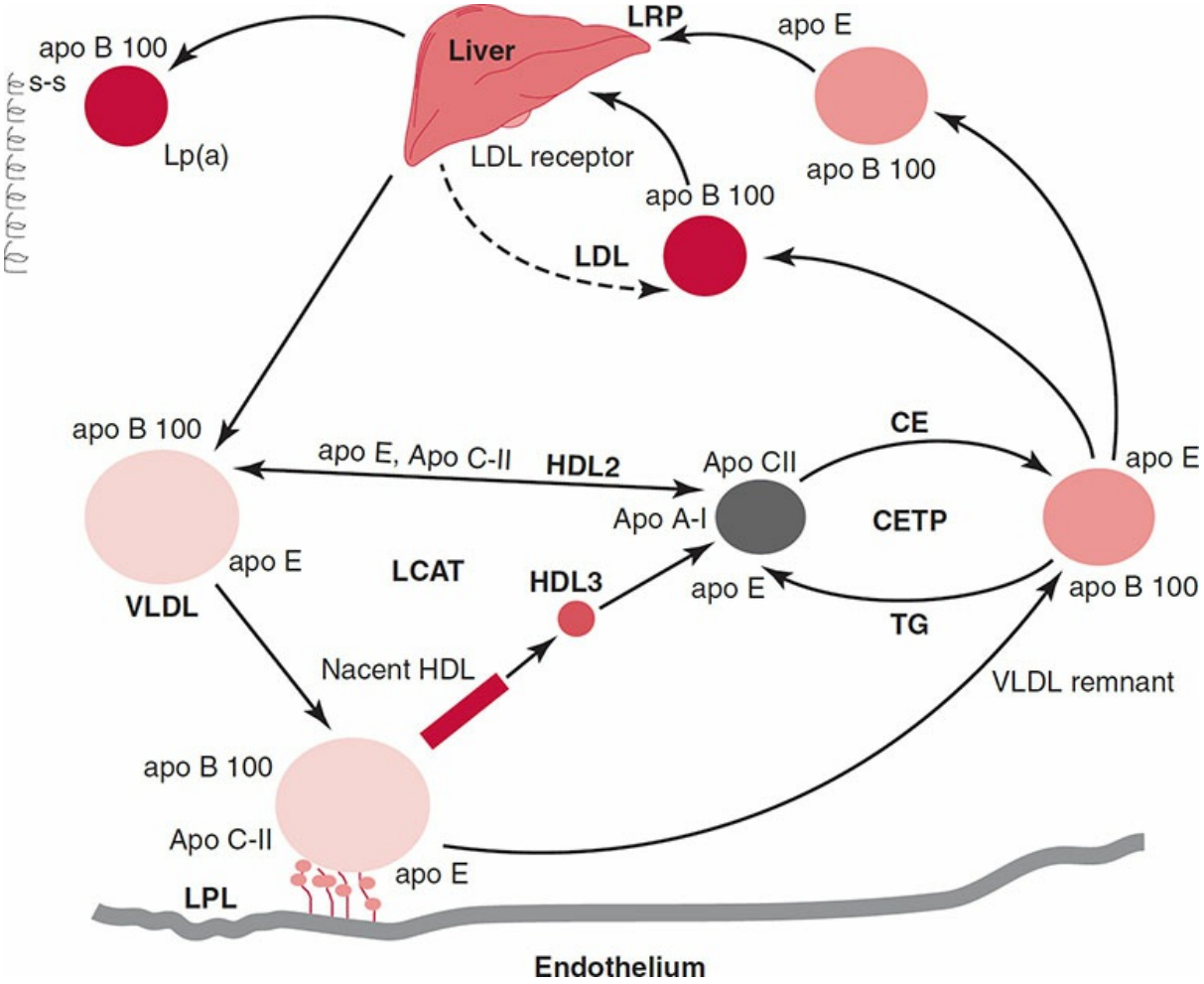

Figure 14–16 Lipoprotein metabolism: very-low-density lipoprotein (VLDL) is secreted by the liver and is hydrolyzed on the vascular endothelium by low-density lipoprotein (LPL). LPL (*small filled circles*) is bound electrostatically to heparan sulfate and, in the presence of apo C-II hydrolyzes triglycerides (TG) releasing free fatty acids, monoglycerides, and diglycerides for cellular uptake. Other surface constituents of VLDL, free cholesterol, and phospholipids participate in the formation of nascent HDL. The free cholesterol on the surface of nascent HDL is esterified by the action of lecithin cholesterol ester transferase (LCAT) to produce cholesterol esters. These sink into the core as nascent HDL is metabolized to the small, dense HDL_3, and finally into the cholesterol ester (CE)-rich HDL_2. The relatively TG-depleted VLDL remnant particle is released from the endothelial surface and is then either taken up by the liver directly via the remnant receptor, which recognizes apo E, or interacts with CE-rich HDL_2. In that interaction, catalyzed by cholesterol ester transfer protein (CETP), the CE-rich core of HDL_2 is exchanged for the TG-rich core of the VLDL remnant, yielding a TG-rich HDL molecule (data not shown) and LDL, which is then taken up by the LDL receptor in the liver, which recognizes apo B 100, the isoform secreted by the liver. HDL_2 is processed by lipases to HDL_3 to continue the cycle. LDL arises from delipidation of VLDL but its rate of synthesis in the nephrotic syndrome may be greater than that of VLDL, suggesting direct secretion from the liver also may occur. Likewise Lp(a), which contains a molecule of LDL, has an increased synthetic rate in the nephrotic

syndrome and also may be secreted directly.

Although this does not explain the increase in LDL and Lp(a), it does nicely close the circle among urinary protein losses, hypoalbuminemia, and decreased lipoprotein catabolism.

Studies in patients with the nephrotic syndrome have not been as detailed as in the rat; however, both species exhibit similar disturbances in lipid metabolism when comparable studies are evaluated. The fractional turnover rate of TGs is reduced in nephrotic subjects compared with controls, and the half-life of TG is prolonged from 4 to 11 hours in VLDL (242). Not only is VLDL catabolism decreased, but the disappearance curve has an unusual shape presumed to result from a delay in the conversion of VLDL to IDL. The delay in lipolysis in humans, as in rats, is proposed to result from a decrease in LPL activity. Evidence supporting this hypothesis is that LPL activity is reduced in children with the nephrotic syndrome and increases after remission. Furthermore, there is a strong inverse correlation between LPL and the concentration of TGs in the VLDL fraction (243).

Mechanism of Reduced LDL Clearance

A marked reduction in LDL receptor (LDLR) protein has been noted in the livers of rats with the nephrotic syndrome (244). The LDLR levels are regulated, at least in part, by proprotein convertase subtilisin/kexin type 9 (PCSK9), a primarily liver-derived protein that binds to the LDLR and induces its internalization and degradation by hepatocytes (245,246). Plasma PCSK9 levels are markedly increased in serum in nephrotic rats (244) and patients (247), despite being of molecular weight (72 kDa) that should result in increased urinary losses (248). The mechanism of increased production of PCSK9 is increased synthesis driven by augmentation in hepatic gene expression, similar to what occurs with regard to albumin, fibrinogen, and other liver-derived proteins (103). When proteinuria is reduced in patients with the nephrotic syndrome, blood lipid levels decrease (131) even if plasma albumin concentration or *p* is unchanged, suggesting that proteinuria plays a role independently of plasma albumin concentration in the nephrotic syndrome in humans as well as in experimental models of the nephrotic syndrome in animals.

Increased Lipoprotein Synthesis

The second set of disorders in lipid metabolism, although well characterized with regard to how they affect lipid levels, is less well understood with regard to pathogenesis. Synthesis of both LDL and Lp(a) is increased. Synthesis of the apo B 100 molecule in LDL does not correlate with that of albumin and with the other serum proteins whose rates of synthesis correlate with one another in the nephrotic syndrome and is likely regulated by a different mechanism. By contrast, Lp(a) synthesis is greatly increased. Its plasma levels correlate directly with its rate of synthesis, independent of isoform (132) (Fig. 14-12). Unlike VLDL, its fractional rate of catabolism is unchanged, and its levels are controlled entirely by an increase in synthetic rate.

Perhaps of more basic interest is the observation that synthesis of apo B 100 in LDL is greater than that in VLDL in some patients. This is in contradiction to the standard model of lipoprotein production that holds that VLDL is released by the liver and serves as the precursor of LDL. The observations of de Sain suggest that at least some component of the LDL pool bypasses this classic delipidation pathway. Similarly, synthesis of apo(a) and apo B 100 in LP(a) occurred at the same rate, suggesting that in the nephrotic syndrome, apo(a) and apo B 100 are synthesized simultaneously from the same precursor pools of amino acids, are linked in the liver, and are secreted by a pathway that is independent of the LDL and/or VLDL pathway(s).

Changes in the Activities of Liporegulatory Enzymes and of Lipoprotein: Clinical Implications of Hyperlipidemia in Renal Disease

The changes that occur in blood lipoprotein composition in the nephrotic syndrome (133)—reduced HDL_2 cholesterol, a relative increase in HDL_3 cholesterol, and the massive increase in total cholesterol, mostly found in the LDL, IDL, and VLDL fractions—should be expected to cause increased risk of atherosclerotic disease, as should the increase in Angptl4. These abnormalities are further complicated by the increase in plasma Lp(a) levels, increased platelet aggregability (249), increased plasma viscosity, increased concentration of highly atherogenic remnants of VLDL, and CM catabolism in plasma. Indeed, accelerated atherosclerosis

has been reported in patients with proteinuria and hyperlipidemia and in some studies has been associated with a sharply increased incidence of cardiovascular disease and stroke (250). One study reported an 85-fold increase in the incidence of ischemic heart disease in such patients (251). In another recent retrospective analysis of 142 patients with proteinuria >3.5 g/day, the relative risk of myocardial infarction was found to be 5.5 and the risk of cardiac death 2.8 compared with age-matched, sex-matched controls (252). However, no randomized prospective trials have been published that provide evidence that lipid-lowering therapy provides an improvement in outcome (253).

Disordered lipid metabolism has been hypothesized to play a role in the cycle of progressive renal failure that occurs following the initiation of renal injury (254), although again this link has by no means been established in humans or in animal models of renal disease that are not associated with substantial increases in cholesterol levels. Indeed, evidence-based data derived from a double blind randomized prospective trial of lowering LDL cholesterol with a statin was negative (255) suggesting no causal link. However, one disorder that causes hypercholesterolemia in humans, hereditary LCAT deficiency, may be linked to progressive mesangial and glomerular sclerosis (256), although this relationship cannot be generalized to other causes of disordered lipid metabolism.

Treatment of Hyperlipidemia

It is not indicated to treat the qualitative abnormalities that characterize the lipid disorders of the nephrotic syndrome or to treat hyperlipidemia if the underlying cause of the nephrotic syndrome is directly treatable, such as in minimal change nephrotic syndrome. If, however, the duration of hyperlipidemia is anticipated to be prolonged, it is wise to initiate therapy. The first goal of treatment, however, should be to reduce urinary protein excretion, if possible. Treatment of nephrotic patients with either ACEIs (154), ARB (257–259), or cyclooxygenase inhibitors (260) results in a decline in both proteinuria and blood lipid levels even if plasma albumin concentration does not increase or increases only slightly (261). Treatment of ACE inhibition has been shown to increase glomerular size selectivity in membranous glomerulopathy, suggesting structural rather than just hemodynamic changes are effected by reduction in activity of the renin–angiotensin axis (262). The addition of the renin inhibitor aliskiren to ARB

therapy has an additive effect on reduction in urinary protein (263). The decline in blood lipid levels includes a decrease in total cholesterol, Lp(a), VLDL and LDL cholesterol, and the activities of cholesterol ester transfer protein (CETP) and LCAT (261). The effect of ACEIs is a class effect and appears to be shared by all drugs within this class.

It is probably prudent to restrict dietary cholesterol and saturated lipids in patients with the nephrotic syndrome. If conservative therapy (reduction in proteinuria, dietary fat restriction) does not effectively reduce hyperlipidemia, a variety of lipid-lowering drugs, including the 3-hydroxy-3-methyglutaryl coenzyme A reductase (HMG CoA reductase) inhibitors (264), antioxidants (265), and fibric acid derivatives (266) can be useful, but a review of this subject is beyond the scope of this chapter.

Edema Formation: Defenses Against Reduced Plasma p

Edema formation in the nephrotic syndrome is a consequence of two processes that act coordinately to increase interstitial fluid volume (267)—one a consequence of decreased oncotic pressure decreasing the retention of fluid within the capillary space driven by capillary hydrostatic pressure, and the second a consequence of primary renal fluid retention as a consequence of presentation of protein to the renal distal tubule (268). Capillary hydrostatic pressure serves to force fluid from the vascular compartment into the interstitial space. This hydrostatic force is partially balanced by the difference between plasma *p* and that exerted by interstitial proteins. Interstitial protein concentration is between 25% and 50% that of plasma protein (269) and the difference between *p* exerted by plasma and interstitial proteins (Δp) serves to retain salt and water in the vascular space. Fluid not reabsorbed by the time blood has reached the venous end of the capillary bed and is returned to the vascular space via the lymphatics.

In steady state:

$$\text{Lymph flow} = K_f\,[(\text{Capillary hydrostatic pressure} - \text{tissue hydrostatic pressure}) - (\text{plasma } \pi - \text{tissue } \pi)] = K_f(\bar{\Delta P} - \bar{\pi}) \quad (14.1)$$

where K_f is capillary hydraulic conductivity (270,271).

When the fall in Δp becomes great enough, the net amount of fluid filtered by the capillaries will exceed maximal lymph flow and edema will

inevitably occur. This increased net transport of fluid into the interstitial space should lead to plasma volume contraction. The plasma volume contraction activates the renin–angiotensin–aldosterone axis, the sympathetic nervous system, and other neurohormonal systems, leading to secondary renal sodium retention. This is the so-called “underfill” model of edema formation. However, Meltzer et al. (272) subsequently identified a group of patients with the nephrotic syndrome who had a normal or an expanded plasma volume and reduced plasma renin activity despite a profound reduction in *p*. These patients represented a subset that had nephritic disease as opposed to patients with minimal change nephrotic syndrome. Some patients with minimal change nephrotic syndrome also have been found to have an increased plasma and blood volume (273,274) as evidenced by the fact that plasma volume actually decreased when patients with minimal change nephrotic syndrome entered remission. Although activation of the renin–angiotensin axis can be found to play a role both in establishing blood volume and in renal sodium retention in some subjects, especially when plasma albumin concentration is significantly depressed (275), this is by no means the only or even likely the primary mechanism responsible for edema formation.

How is it possible to maintain a normal or even an expanded plasma volume when *p* is greatly reduced? If it is indeed possible to maintain a normal plasma volume, why does the kidney retain salt and water in nephrotic patients? The answer to the first question lies in part in the fact that interstitial albumin mass is reduced to an even greater extent than is the plasma albumin mass in the nephrotic syndrome (111). The mobilization of extravascular albumin is a rapid, hemodynamically mediated response to a decrease in plasma *p* or to an increase in transcapillary hydrostatic pressure (270–272,276). Interstitial albumin concentration decreases in parallel to plasma albumin concentration following the onset of proteinuria in rats with an experimental form of the nephrotic syndrome induced by injection of puromycin aminonucleoside (277). Although albumin decreases, Δp decreases little or not at all unless albumin concentration decreases below 2 g/dL because interstitial protein is swept into the vascular compartment by increased lymphatic flow. In addition, because the capillary endothelium is far more permeable to water than to protein, when transcapillary hydraulic flux increases, the resulting plasma ultrafiltrate is much poorer in protein than when hydraulic flux is reduced. Δp does not decrease in nephrotic rats until saline is administered resulting in volume expansion.

Edema formation in the nephrotic syndrome may involve two parallel

processes (Fig 14-17). Reduced plasma *p* leads to augmented net flux of fluid across the systemic capillary bed (underfill), but these alterations may be entirely or largely offset by increased lymphatic return from the periphery and reduction in interstitial *p* so that Δp remains unchanged. Edema formation is not generally obligated to occur until total proteins decrease to around 4 g/dL. The second process results from a primary impaired ability of the nephrotic kidney to excrete a sodium load, either in response to plasma volume expansion (267,278,279) or in response to atrial natriuretic peptide (ANP) (268,280). Additionally, there is decreased conversion of proANP, the form secreted in response to volume expansion, to the active form of ANP because of downregulation of corin, the protease responsible for this conversion (281), in the kidney (282). This primary renal retention of sodium seen in the nephrotic syndrome is sometimes referred to as the "overflow" model of edema formation. A number of mechanisms have been proposed to account for this primary renal sodium retention. First, there may be increased activity of the Na^+/K^+ ATPase pump in the cortical collecting duct (283). NO synthase activity is also impaired (284). The resistance to ANP is mediated at least in part by an increase in cyclic GMP phosphodiesterase activity in the distal nephron (285). This enzyme leads to degradation of the second messenger of ANP, cyclic GMP. Thus, increased activity of phosphodiesterase in the setting of nephritic syndrome will blunt natriuresis. Studies using unilateral models of proteinuria most clearly demonstrate that impedance to sodium excretion is intrinsic to the nephrotic kidney and not reflective of plasma volume regulation. In one such study, Perico et al. (268) demonstrated an inability of a proteinuric kidney to excrete fluid or sodium in response to infused ANP, even though ANP increased GFR in both the proteinuric kidney and the normal contralateral kidney equally. Clearly, both the normal contralateral kidney and the proteinuric kidney were exposed to the same *p* and the same levels of circulating hormones responsible for plasma volume regulation. Proteinuria has been shown to inhibit sodium transporters in the proximal nephron as well (286).

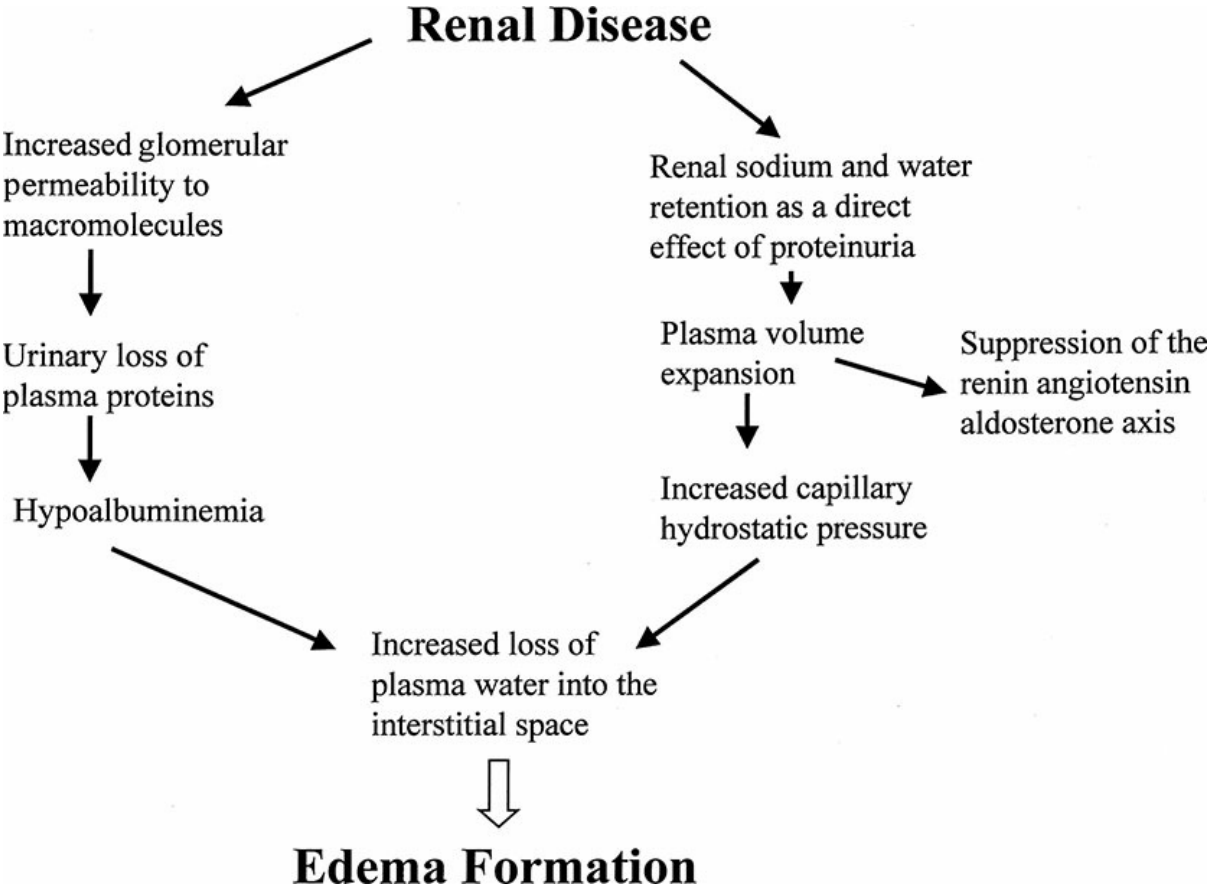

Figure 14–17 Primary renal sodium retention with resultant edema formation: renal salt and water retention occurs as a result of the renal disease itself and causes plasma volume expansion. This produces increased capillary hydrostatic pressure, which in conjunction with the increased transcapillary flux of fluid resulting from hypoalbuminemia causes edema. Edema formation is not a direct consequence of reduced *p* alone, nor is renal salt and water retention a consequence of increased transudation of fluid into the interstitial space with resultant activation of the renin–angiotensin–aldosterone axis. (From Kaysen GA, Myers BD, Couser WG, et al. Mechanisms and consequences of proteinuria. *Lab Invest*. 1986;54:479–498, with permission.)

Regardless of the mechanism, the proteinuric kidney avidly reabsorbs filtered salt in the distal nephron (287) even in the presence of plasma volume expansion and reabsorption of sodium in the proximal nephron is impaired as well. As a consequence of these combined effects, the systemic capillary bed is faced with increased hydrostatic pressure at the very time that defense mechanisms normally employed to counteract edema formation, increased lymphatic flow, and decreased interstitial protein concentration already have been maximized. Edema results from the combined effect of primary renal salt and water retention coupled with reduced defenses against edema formation resulting from the urinary losses of proteins of intermediate weight with the resulting decrease in

both plasma and interstitial *p*. These processes deprive the lymphatic system of the capacity to respond to increased hydrostatic pressure.

Nutritional Recommendations

DIETARY PROTEIN

In patients with the nephrotic syndrome, dietary protein augmentation above 1 g/kg/d is not of demonstrated benefit and may cause increased urinary albumin losses (95,288). Dietary protein augmentation may actually result in a decrease in serum albumin concentration because any augmentation in albumin synthetic rate is more than accounted for by increased urinary albumin excretion (134,135). These patients' diets should provide between 0.8 and 1.0 g/kg/d of protein and 35 kCal/kg/d of energy. Twenty-four-hour urinary protein excretion should be measured every 2 to 3 months and urinary urea excretion monitored to ensure that patients are eating the quantity of protein recommended, and that proteinuria decreases and albumin and protein concentration does not decrease when dietary protein intake is restricted to these levels.

Dietary protein intake can be estimated because in steady state, dietary protein is equal to the protein catabolic rate. If total body urea pools do not change (blood urea nitrogen is neither decreasing nor increasing), it is possible to estimate the amount of protein that has been eaten by the formula:

$$\text{Protein catabolic rate} = (10.7 + \text{24-hour urinary urea excretion}/0.14)\ \text{g/day} + \text{urinary protein excretion} \quad (14.2)$$

An accurate nutritional history should be obtained and the diet adjusted accordingly if there is variance from the prescribed diet.

DIETARY FAT

The mechanisms establishing disordered lipid metabolism in the nephrotic syndrome have been discussed above and are primarily driven by the reduced catabolic rate associated with proteinuria with an additional contribution of increased synthesis of Lp(a) and apo B (132). Soy or vegan diets have been shown to reduce urinary protein excretion in patients with the nephrotic syndrome (289). Although it is claimed that this effect is a consequence of the reduced lipid content of the diets, there are no

convincing data presented that changes in dietary lipids are responsible for the salutary effects of these diets. Lipids, however, represent a wide variety of substances, including steroids, saturated and unsaturated fatty acids, phospholipids, and other compounds, many of which are either directly biologically active or precursors of important biologically active metabolites. Much attention has been focused on the effect of polyunsaturated fatty acids on renal hemodynamics and on expression or renal injury.

In studies involving human subjects, Gentile et al. (289,290) added 5 g of fish oil per day to the diet of patients with the nephrotic syndrome who had been maintained on a soy vegetarian diet, and found no beneficial effect on either proteinuria or on blood lipids compared with patients maintained on the soy diet without fish oil supplementation. In contrast, Hall et al. (291) found that 15 g of fish oil per day caused a decrease in total TGs and in LDL TGs with an increase in LDL cholesterol. Donadio et al. (292) treated 55 patients with IgA nephropathy with 12 g of fish oil per day in a prospective randomized placebo-controlled study and found a significant reduction in the rate of progression of renal disease using a 50% increase in serum creatinine concentration as a study end point. At the end of the treatment period, the fish oil–treated group had a lower prevalence of hypertension, elevated serum creatinine, and nephrotic range proteinuria. The rate of progression to end-stage renal disease was reduced by fish oil supplementation in patients with IgA nephropathy; however, the effect was not dose dependent (293). The salutary effect cannot be extended necessarily to other causes of the nephrotic syndrome and at this point should be considered as part of the specific treatment recommendations for IgA nephropathy. Not all investigators have demonstrated a positive effect of these agents even among subjects with IgA nephropathy (294). These agents may be neither predictable nor salutary for all patients with renal disease.

Pharmacologic Means to Reduce Glomerular Proteinuria

RENIN/ANGIOTENSIN BLOCKADE

ACEIs and ARBs (295) reduce proteinuria in experimental models of the nephrotic syndrome and in some nephrotic patients as well. ACEIs may reduce proteinuria by a number of mechanisms. Angiotensin II receptor

blockage specifically prevents the reduction in the expression of mRNAs encoding both nephrin and NEPH1 that characterize damage to the slit diaphragm in experimental nephrotic syndrome in the rat model of puromycin aminonucleoside nephrotic syndrome (296) suggesting processes beyond hemodynamic effects. Both losartan, and ARB, and captopril, an ACEI, reduce proteinuria in a diabetic rat model; however, only captopril preserves basement membrane anionic charge, suggesting that the mechanisms of the two classes of agents may differ (297). Dual blockade by the combined use of an ACE and ARB appears to be more effective at reducing urinary protein excretion than is increasing the dose of one of the agents, possibly depending upon the causative pathology (298–300), although this additive effect may depend upon the underlying pathology, and be less effective in some disease processes, such as diabetic nephropathy (301,302), although some studies have found a small additive effect even among diabetic subjects (303). Use of both classes of drugs together can be limited by safety issues (304). Triple therapy, with the addition of an aldosterone antagonist, may also increase efficacy over that seen with an ACE plus an ARB in nondiabetic patients (305). The combination of low-dose ARB and ARB has been shown in some studies to be more effective than higher doses of single agents (306,307). Reduction in proteinuria with these agents reduces blood lipid levels as expected (308); however, while addition of a statin further reduces blood lipids, the addition of a statin has no further effect on urinary protein loss.

The addition of the renin antagonist aliskiren to block activation of angiotensin II, has not shown additional benefit over either ACE or ARB monotherapy, and additionally has raised safety concerns (309–311).

By contrast, the addition of a mineralocorticoid receptor antagonist may provide additional benefit with regard to reducing urinary protein excretion (312,313) most likely by reducing fibrosis of the renal interstitium promoted by aldosterone rather than by hemodynamic effects. Caution must be used when adding a mineralocorticoid antagonist to either an ACE or ARB or to both because of the predictable increase in serum potassium that is likely to occur (314).

Endothelin antagonists also have demonstrated efficacy in reducing urinary protein losses as well as progression of renal injury (315). Ang II is a growth factor; thus, diminishing its production or action may reduce glomerular hypertrophy. Moreover, as Ang II stimulates mesangial matrix formation and fibrosis, in part by enhanced release of transforming growth factor-β and other growth factors, blockade or reduction in Ang II activity may attenuate these profibrotic forces in glomerular disease (316,317)

primarily effected by aldosterone and endothelin (318,319). A reduction in mesangial expansion, glomerular hypertrophy, and fibrosis by Ang II blockade may contribute to the reduction in proteinuria. Many glomerular diseases such as diabetes nephropathy are associated with increased podocyte loss and apoptosis and decreased nephrin gene expression. Ang II blockade attenuates these effects on the podocytes (320,321).

GFR is generally not reduced by ACEIs or ARBs. Proteinuria is reduced, accompanied by an increase in serum albumin concentration and a reduction in blood lipid levels in rats with experimental models of the nephrotic syndrome and in patients with the nephrotic syndrome (322,323).

The antiproteinuric effect of ACEIs and ARBs seems to be shared by all drugs of these classes studied to date; however, this property is not shared by all other antihypertensive agents. Results obtained with both α antagonists and certain classes of calcium channel blockers have been inconsistent and not sufficiently well documented to warrant clinical use of these agents for control of proteinuria at this time. Dihydropyridine calcium channel blockers may actually increase albuminuria (324). Decreased urinary protein excretion occurs gradually over 1 or 2 weeks. In some instances, neither the blood pressure nor the proteinuria responds to rather large doses of ACEIs and therapy with these agents should then be discontinued. Some patients, especially those with renal artery stenosis, may exhibit a marked reduction in blood pressure and in GFR with even low doses of ACEIs. It is important to identify these patients as well and discontinue ACEI therapy if a marked reduction in GFR occurs.

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CHAPTER 15

The Glomerulopathies

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Disorders affecting the structure and function of the glomeruli (glomerulopathies) are among the most common causes of acute and chronic renal insufficiency. Broadly defined, the glomerulopathies include diseases that may originate in the glomerular capillaries, the glomerular basement membrane (GBM), the mesangium, the podocyte, or outside the glomerular tuft. Such a definition includes diverse diseases such as immune-mediated glomerulonephritis (GN), diabetic kidney disease, and thrombotic microangiopathies. Some of these diseases present as primary diseases of the kidney, and others, such as diabetic nephropathy (DN), represent the renal manifestation of a systemic disorder.

Classification of the glomerular diseases can be complex because the definition of these diseases incorporates etiology, pathogenesis, histologic findings, and clinical syndromes. Disease classification is particularly complex for the different types of GN (Table 15-1). The definition of these diseases relies heavily upon the histologic findings. As more information about the etiology and pathogenesis of glomerular diseases has become available, however, it has enabled more precise subclassification of some disease processes. Patients who previously would have been given the diagnosis of focal segmental glomerulosclerosis (FSGS) or membranoproliferative glomerulonephritis (MPGN) based on the histologic appearance seen on renal biopsy, for example, may now be further subclassified on the basis of genetic factors or infectious etiologies.

Table 15–1 Types of Glomerulonephritis

Disease	Common Clinical Presentation
IgA nephropathy and Henoch–Schönlein purpura	Microscopic hematuria, subnephrotic proteinuria
Lupus nephritis	Microscopic hematuria, proteinuria, nephritic syndrome, nephrotic syndrome
Membranoproliferative glomerulonephritis	Nephritic syndrome
C3 glomerulopathy (dense deposit disease and C3 glomerulonephritis)	Nephritic syndrome
Cryoglobulinemia	Nephritic syndrome
Infection-associated glomerulonephritis	Nephritic syndrome
Minimal change disease	Nephrotic syndrome
Focal segmental glomerulosclerosis	Nephrotic syndrome
Membranous glomerulonephritis	Nephrotic syndrome
Amyloidosis	Nephrotic syndrome
Fibrillary/immunotactoid glomerulonephritis	Nephrotic syndrome
Granulomatosis with polyangiitis	Rapidly progressive glomerulonephritis
Microscopic polyangiitis	Rapidly progressive glomerulonephritis
Goodpasture disease	Rapidly progressive glomerulonephritis

It should be emphasized that unique pathogenic events can give rise to diverse morphologic features and a single morphologic pattern can evolve from several pathogenic mechanisms. Furthermore, discrete morphologic abnormalities can evoke a spectrum of clinical syndromes. Refining the definition of a disease as new discoveries are made is important insofar as it may offer more accurate prognostic information and may allow

clinicians to choose the most appropriate treatment for a given patient. Recent insights into the pathogenesis of C3 glomerulopathy (C3G), for example, have led to the reclassification of patients who previously would have been diagnosed with MPGN (1). These diseases originally bore similar names because of their histologic appearance by light microscopy, but C3G is now classified based upon the underlying immunologic mechanisms of injury. In the future, genomic, transcriptomic, proteomic, and metabolomic studies will undoubtedly lead to more precise molecular classifications of the glomerular diseases (2). This, in turn, will allow mechanism-based diagnoses, more accurate prognostic information, and may lead to therapies that specifically target the underlying causes of disease.

Etiology

Glomerulopathies can arise from the effects of environmental agents (microbial infection, drugs, or toxins) or from endogenous perturbations (altered metabolism, biochemical defects, autoimmunity, or neoplasia). In both instances, underlying genetic factors interact with the environmental agents to generate the morphologic and clinical expression of disease. The etiologic agent responsible for the glomerular disease is well understood in some circumstances (e.g., drug-induced membranous nephropathy [MN]), although the pathogenic mechanisms responsible for the disease are unknown. For others, the etiologic agent remains obscure even though the effector systems engaged in tissue injury are reasonably well known. The search for the etiologic entities involved in the glomerulopathies continues.

Clinicopathologic Patterns of Injury

The glomerulus is a highly organized structure, and immunologic glomerular injury generally falls into one of several morphologic patterns. Patients with glomerular disease also tend to present with clinical findings that fit into one of several syndromes. Diseases of varying etiologies may cause glomerular injury with similar morphologic and clinical findings. Conversely, patients with the same underlying disease, such as systemic lupus erythematosus (SLE), may present with different clinical and pathologic patterns of injury. In general, the clinical findings are

insufficient to accurately diagnose and treat glomerular diseases and a renal biopsy is necessary. Renal biopsies are processed for evaluation of the tissue by light microscopy, electron microscopy (EM), and immunofluorescence microscopy in order to fully classify the morphologic features of the disease. Various special stains can also be used to distinguish particular diseases.

The glomerular diseases are associated with several clinical syndromes. The nephrotic syndrome (see also Chapter 14) is usually defined as >3 to 3.5 g of proteinuria per day. Patients with the nephrotic syndrome have hypoalbuminemia, edema, and hypercholesterolemia. The urine of patients with the nephrotic syndrome does not typically contain dysmorphic red blood cells or red blood cell casts. Patients with the nephrotic syndrome are at increased risk of venous thrombosis and infection. Diseases causing subnephrotic range proteinuria (<3 g of proteinuria per day) may pose a significant threat to the patient, but they are less likely to develop symptoms of the nephrotic syndrome. The nephritic syndrome often refers to disease presenting with hematuria, proteinuria, and dysmorphic red blood cells or red blood cell casts. Proteinuria is usually present, and can range from subnephrotic levels to >10 g/day. Depending upon the extent of glomerular involvement, patients may develop hypertension, edema, and/or renal insufficiency. As will be discussed in detail later, there is great heterogeneity in the presentation of the glomerular diseases. Thus, disease etiologies commonly described as presenting with one of these syndromes may present with variable constellations of signs and symptoms.

IMMUNE COMPLEXES

Numerous cellular and molecular mechanisms of glomerular injury have been identified, but immune complex (IC)–mediated injury warrants additional attention as the glomerulus is a common site for IC deposition (Table 15-2). The size and charge of ICs is a function of several factors, including the nature of the antigen, the isotype of the antibodies and their affinity for the antigen, and the relative abundance of antigen and antibody. Circulating ICs may be trapped in the glomerulus, a process that could be enhanced by the large flow of plasma through the kidneys, high intraglomerular pressures, permeable capillary walls, and an anionic basement membrane that can bind cationic antigens (3). Circulating ICs will tend to accumulate in the mesangium and subendothelial space as they are too large to pass through the GBM. ICs may also form in situ when

antibodies bind to antigens that are expressed within the glomerulus or that become trapped there. For example, antibodies to the M-type phospholipase A_2 receptor (a protein present on podocytes) are present in approximately 70% of patients with idiopathic MN (4). In situ IC formation may lead to deposition between the GBM and the podocytes, and identification of the antigen in various IC-mediated renal diseases is instrumental for understanding the disease process.

ICs trigger the generation of several pro-inflammatory factors. They activate complement, thereby generating C5a and the membrane attack complex. They also trigger the release of numerous other pathologic factors by resident or infiltrating cells, including chemokines, prostaglandins, platelet-activating factor, procoagulant factors, and adhesion molecules. Many of these molecules are involved in leukocytes trafficking to the site of inflammation, and infiltrating polymorphonuclear neutrophils (PMNs), macrophages, and T cells then release additional factors that also contribute to tissue injury.

- *Mesangial IC deposition—mesangial expansion—hematuria and proteinuria*. Several diseases, such as immunoglobulin A nephropathy (IgAN) and lupus nephritis, are associated with the deposition of ICs in the mesangium (Fig. 15-1). Mesangial IC deposition is associated with the development of hematuria and proteinuria, but nephrotic range proteinuria and significant changes in the glomerular filtration rate (GFR) are not typically seen. Injury of the mesangial cells stimulates the cells to proliferate and to produce extracellular matrix. Over time, this can lead to glomerular sclerosis and irreversible renal injury.
- *Subendothelial IC deposition—endovascular proliferation—nephritic syndrome*. In diseases such as lupus, postinfectious GN, and MPGN, ICs deposit in the subendothelial space (Fig. 15-2). The complement activation fragments and inflammatory factors generated at this location have access to the circulation and tend to cause an inflammatory lesion. Subendothelial ICs cause endocapillary proliferation, where the glomerular capillaries appear hypercellular and are filled with inflammatory and endothelial cells. Clinically, patients with these diseases commonly present with the nephritic syndrome. The extent of glomerular involvement tends to correlate with disease severity (e.g., diffuse involvement causes a greater decline in GFR than focal), but clinical parameters are an unreliable guide to the severity of the underlying lesion. Antibodies may be

pathogenic in diseases in which ICs are not seen in the kidney biopsy. In small vessel vasculitis, for example, patients may present with a nephritic syndrome. Although they can appear "pauci-immune" on biopsy, antibodies probably play a critical role in the pathogenesis of these diseases (vide infra). The thrombotic microangiopathies may also cause endothelial injury and present with signs of acute GN.

Table 15–2 Frequently Seen Clinicopathologic Correlations in Patients with Glomerulonephritis

Pathologic Findings	Syndrome	Diseases
Mesangial immune complexes/mesangial proliferation and expansion	Microscopic hematuria, subnephrotic proteinuria	IgA nephropathy, lupus nephritis
Subendothelial immune complexes/endocapillary proliferation	Nephritic syndrome	MPGN, lupus nephritis
Subepithelial immune complexes/thickened appearance of glomerular basement membrane	Nephrotic syndrome	Membranous disease, lupus nephritis
Glomerular crescents. May be associated with linear Ig deposited along the GBM, pauci-immune GN, or subendothelial ICs.	Rapidly progressive glomerulonephritis	Goodpasture disease, ANCA associated vasculitis lupus nephritis, MPGN

IgA, immunoglobulin A; MPGN, membranoproliferative glomerulonephritis; GBM, glomerular basement membrane; GN, glomerulonephritis; IC, immune complex; ANCA, antineutrophil cytoplasmic antibody.

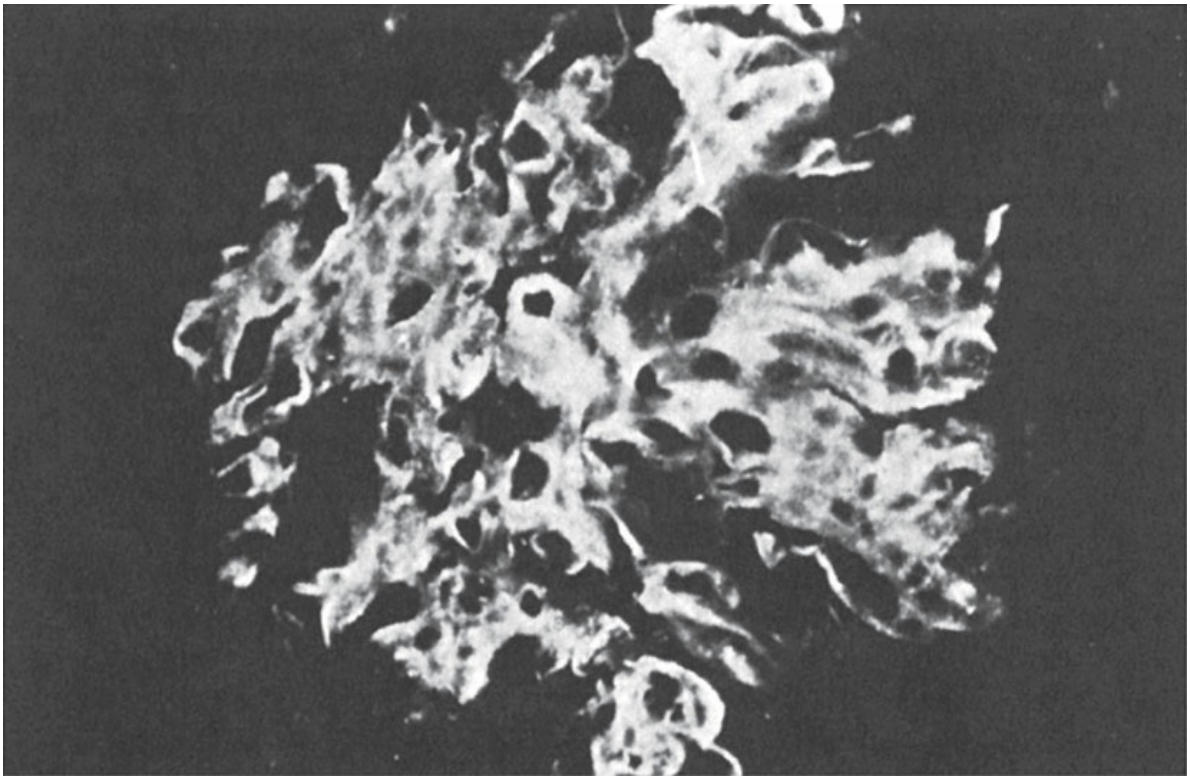

Figure 15–1 Fluorescent antibody staining, showing diffuse, coarse immunoglobulin deposits located predominantly in the glomerular mesangium. This finding is characteristic of a large group of diseases that often demonstrate focal or diffuse mesangial proliferative glomerulonephritis by light microscopy, including IgA nephropathy.

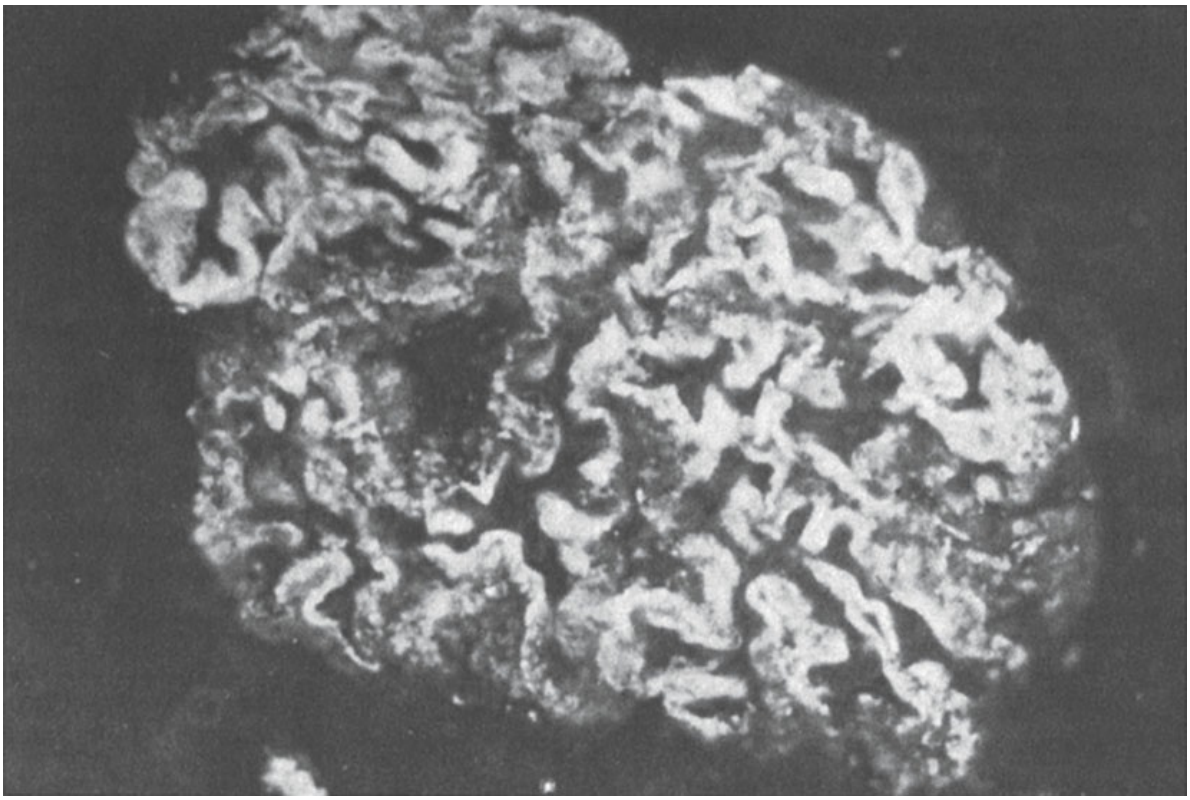

Figure 15–2 Fluorescent antibody staining of the glomerulus, demonstrating discrete granular deposition of immunoglobulin along the basement membrane, which is characteristic of immune complex–mediated glomerulopathies. The immune complexes contained within the granular deposits may have deposited from the circulation *or* formed locally (in situ) as a consequence of interaction of a circulating antibody with a native glomerular capillary wall antigen or an extrinsic antigen planted in the glomerulus because of an affinity for constituents of the capillary wall.

- *Subepithelial IC deposition—podocyte injury—nephrotic syndrome*. The podocyte is a highly differentiated cell that forms an important part of the glomerular filtration apparatus. The podocyte has branches that terminate in foot processes that are anchored to the GBM. The foot processes of adjacent cells interdigitate and are separated from each other by a slit diaphragm. ICs that form in a subepithelial location (i.e., between the podocyte and the GBM) can injure the podocyte, disrupt the slit diaphragm apparatus, and cause foot process effacement. Clinically, patients with subepithelial ICs present with the nephrotic syndrome and with a membranous pattern on biopsy (5). The inflammatory factors generated by subepithelial ICs are predominantly excreted in the urine and do not cause leukocyte infiltration or hypercellularity (Fig. 15-3). Consequently, hematuria, pyuria, and cellular casts will typically not be present.
- *Crescentic renal disease—rapidly progressive glomerulonephritis (RPGN)*. Glomerular crescents are extracapillary aggregates that form in Bowman space and compress the capillary tuft (Fig. 15-4). They are composed of cells (proliferating parietal epithelial cells, infiltrating monocytes, and fibroblasts) and fibrous material. Crescents may be seen in many types of immune-mediated glomerular disease, including anti-GBM disease, IC-mediated GN, and pauci-immune small vessel vasculitis (Table 15-3). It is believed that crescent formation starts with injury of the capillary wall sufficient to allow cells and plasma proteins into Bowman space. Biopsies in which >50% of the glomeruli have crescents are often referred to as “crescentic,” regardless of the disease etiology, and this finding is associated with a rapid loss of renal function, oliguria, and signs of acute GN.

TUBULOINTERSTITIAL FIBROSIS

Tubulointerstitial injury is common in disease processes regarded as primarily targeting the glomeruli. Tubulointerstitial injury often correlates

better with renal function than the glomerular lesion, and the degree of tubulointerstitial fibrosis is also predictive of the long-term outcome in patients with glomerular disease (6). The glomerular capillaries and the peritubular capillaries are arranged as sequential capillary beds. Glomerular diseases can therefore reduce blood flow to the peritubular capillaries. There is also experimental evidence that altered permselectivity at the glomerulus allows molecules into the ultrafiltrate that can harm the downstream tubular epithelial cells, including transferrin and complement proteins. These may be common processes that are important to the progression of glomerular diseases of different etiologies.

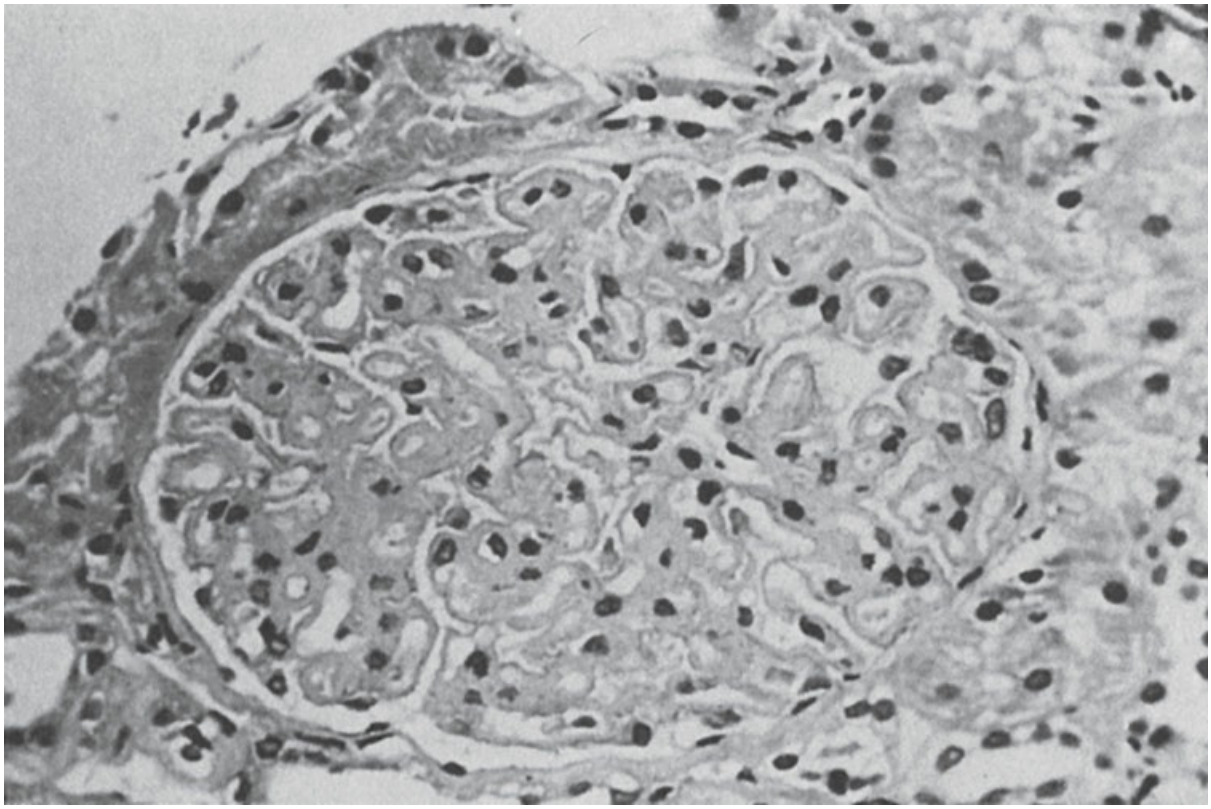

Figure 15–3 Light microscopic appearance of membranous glomerulonephritis. Note diffuse thickening of the capillary walls without associated inflammatory response.

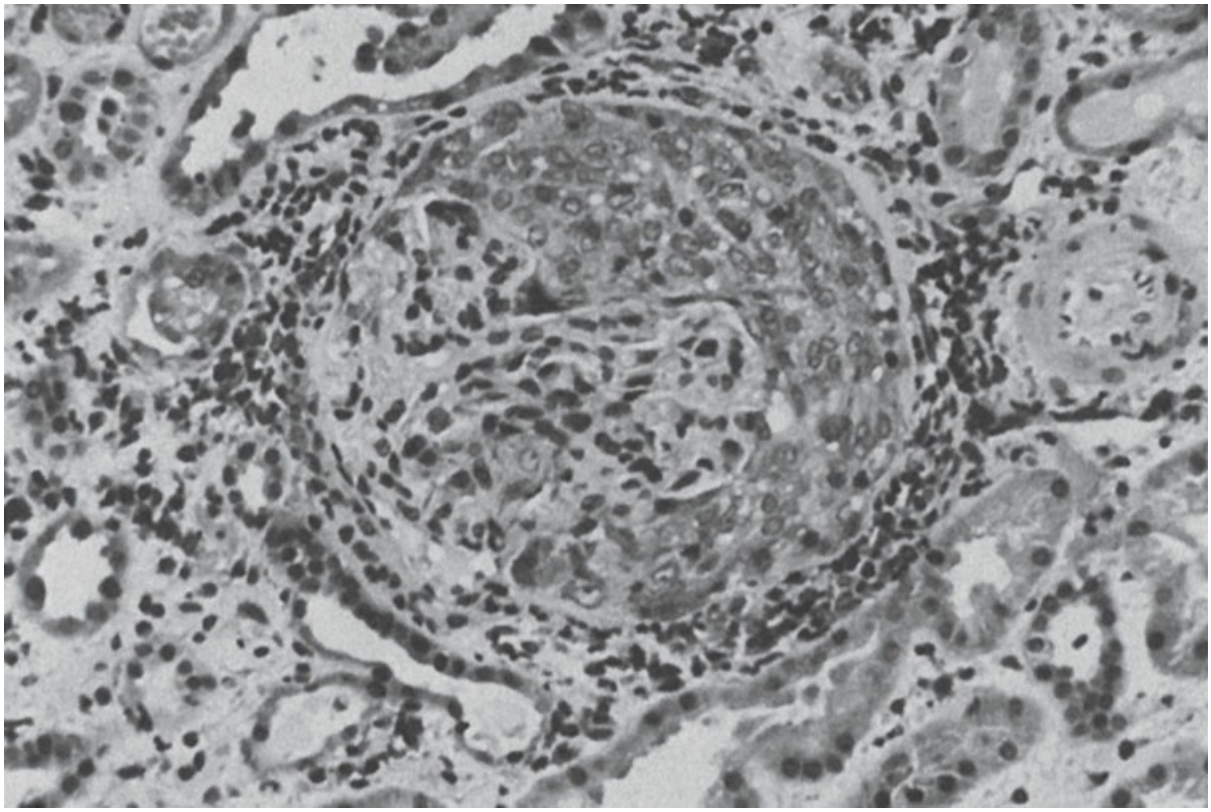

Figure 15–4 Light microscopic findings characteristic of crescentic glomerulonephritis. Note necrotizing glomerulonephritis with marked extracapillary reaction, including crescent and periglomerular fibrosis.

Table 15–3 Causes of Crescentic Glomerulonephritis

Primary	Secondary
	Infectious diseases
Antiglomerular basement membrane antibody mediated	Infective endocarditis, poststreptococcal glomerulonephritis, visceral sepsis, hepatitis B
Immune complex mediated	Multisystem disease
“Pauci-immune” (antineutrophil cytoplasmic antibody associated)	Systemic lupus erythematosus, Goodpasture disease, Henoch–Schönlein purpura, microscopic polyangiitis, Wegener granulomatosis, cryoglobulinemia, relapsing polychondritis, malignancy
Membranous glomerulonephritis	
Membranoproliferative glomerulonephritis	
C3 glomerulopathy	
IgA nephropathy	
	Drug associated
	Allopurinol, rifampicin, D-penicillamine

Important Clinical Scenarios

PULMONARY-RENAL SYNDROME

As will be discussed later, diseases such as Goodpasture disease and granulomatosis with polyangiitis (GPA; formerly known as Wegener granulomatosis) can simultaneously affect the lungs and the kidneys. Severe pneumonia can also present with renal failure, and patients with the sepsis syndrome or congestive heart failure frequently have both pulmonary and renal involvement. Given the importance of early treatment for patients with Goodpasture disease or small vessel vasculitis, however, patients presenting with acute disease of the lungs and kidneys should receive close scrutiny. Proteinuria, an active urine sediment, or serologic evidence of vasculitis may be useful for identifying the underlying etiology, but a renal biopsy may be necessary for definitive diagnosis.

GLOMERULAR DISEASE IN THE CANCER PATIENT

Several types of glomerular disease have been associated with underlying tumors. Overall, the incidence of glomerular disease in cancer patients is rare, but cancer may be found relatively frequently in certain groups of patients with glomerular disease. For example, solid tumors may be found in 23% of patients over 60 years old who are diagnosed with MN (7), and basic cancer screening (e.g., chest X-ray, screening for occult blood in the stool, and colonoscopy) is warranted. A high incidence of solid tumors has also been found in older patients with IgAN (8). Hodgkin disease is associated with minimal change disease (MCD), and non-Hodgkin lymphoma has been associated with several types of glomerular disease including crescentic GN. Monoclonal immunoglobulin deposition diseases of the kidney are frequently caused by underlying lymphoproliferative diseases and are discussed in detail below.

GLOMERULAR DISEASE IN THE PREGNANT PATIENT

The normal pregnancy is associated with several changes in renal physiology (see Chapter 13). Reports have suggested that pregnancy may cause exacerbations of renal disease in patients with chronic GN of several different etiologies. Given the prevalence of lupus nephritis in women of childbearing age, flares of lupus nephritis are relatively common. There are also reports suggesting that pregnancy may cause hypertension or

disease flares in patients with preexisting IgAN, MPGN, and FSGS. In all of these diseases, however, there are conflicting data as to whether pregnancy actually increases the likelihood of a disease flare or whether these reports simply reflect a coincident worsening of disease during pregnancy. The degree of proteinuria often increases during pregnancy, but this is a result of hemodynamic changes and does not indicate a worsening of the renal disease. Regardless of the disease etiology, preexisting hypertension or renal insufficiency are risk factors for complications of the pregnancy. Preeclampsia and HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome are glomerular diseases of pregnancy. These conditions can be difficult to distinguish from the exacerbation of a preexisting glomerular disease, such as lupus. This distinction is made harder by the fact that preexisting renal disease is a risk factor for preeclampsia.

General Treatment Strategies

The glomerular diseases share many pathogenic mechanisms—such as engagement of elements of the innate and adaptive immune systems—and our understanding of the molecular pathogenesis of this diverse group of disorders is constantly growing. Nevertheless, the commonly used therapies usually have broad effects on the overall function of the immune system (Table 15-4). Newer biologic therapies are being developed, however, that offer the possibility of a more targeted approach to the treatment of these diseases. General treatments aimed at controlling the blood pressure and reducing proteinuria are also important for maintaining renal health, even in diseases regarded as being autoimmune in origin.

Primary (Idiopathic) Glomerulopathies

MINIMAL CHANGE DISEASE

MCD, otherwise known as Nil (Nothing-In-Light microscopy) disease or lipoid nephrosis, accounts for 10% to 15% of primary nephrotic syndrome in adults in industrialized countries (9), and it is the most common cause of nephrotic syndrome in children. Experts continue to debate whether MCD is its own disease or whether it exists as part of a continuum with FSGS.

In adults, MCD is diagnosed by kidney biopsy. Few, if any, changes

are seen on light microscopy, and immunofluorescence is also usually unremarkable. EM shows extensive podocyte foot process retraction and effacement. These podocyte changes are not specific to MCD and it is important to exclude other pathologies when making the diagnosis. The lesion of early FSGS may be sparse and usually first appears in deeper glomeruli at the corticomedullary junction. The number of glomeruli obtained by biopsy is important for excluding other diseases. A biopsy with 10 glomeruli has a 35% chance of missing a focal lesion, whereas a biopsy containing 20 glomeruli only has a 12% chance of missing a focal lesion (10). Thus sampling error may miss glomeruli affected by FSGS if <20 cortical glomeruli are obtained. Other rarer disorders may also appear normal by light microscopy, including C1q nephropathy, IgM nephropathy, and idiopathic mesangial proliferative GN. Immunofluorescence typically helps distinguish among these diseases.

Table 15–4 Drugs Commonly Used to Treat Glomerular Diseases

	Proposed Mechanism of Action
Immunomodulatory Agents	
Glucocorticoids	Steroids suppress B-cell and T-cell functions. High doses appear to act in part by inducing the synthesis of IκBa, a protein that traps and inactivates NF-κB thereby decreasing cytokine generation. Steroids may also have cell membrane effects altering the action of membrane-bound proteins and receptors.
Cyclophosphamide	An alkylating agent that covalently binds and crosslinks DNA, RNA, and proteins leading to either cell death or altered cellular function. Cyclophosphamide causes neutropenia and lymphopenia.
Mycophenolic acid	Inhibits T- and B-cell proliferation by blocking purine synthesis via inhibition of inosine monophosphate dehydrogenase.
Cyclosporine/Tacrolimus	Inhibits the phosphatase calcineurin preventing the translocation of nuclear factor of activated T cells leading to reduced transcriptional activation of early cytokine genes. There is evidence that cyclosporine may also stabilize the actin cytoskeleton of podocytes maintaining podocyte function.
Rituximab	Murine/human chimeric anti-CD20 monoclonal antibody that depletes B cells.

Azathioprine	Inhibits T- and B-cell proliferation by blocking purine synthesis.
Chlorambucil	An alkylating agent that crosslinks DNA. It reduces the number of both B cells and T cells.
Plasma exchange	Removes large molecular weight substances—autoantibodies, immune complexes, cryoglobulins, myeloma light chains—from the plasma. When replacement fluid is plasma, allows large volumes of plasma to be infused without the risk of intravascular volume overload.
Eculizumab	Monoclonal antibody to the complement protein C5. It blocks cleavage of C5, thereby preventing the formation of C5 and the membrane attack complex.
Adrenocorticotropic hormone	Mechanism uncertain. May mediate its effects via the α-melanocyte-stimulating hormone and may reduce autoantibody formation.
Nonimmunomodulatory Agents	
ACEI/ARBs	Decrease in intraglomerular pressure. May also have an antifibrotic effect.
Fish oil	Eicosapentaenoic acid and docosahexaenoic acid serve as substrates for cyclooxygenase and lipoxygenase pathways leading to less potent inflammatory mediators than those produced through the arachidonic acid pathway.
Pentoxifylline	Phosphodiesterase inhibitor that inhibits cell proliferation, inflammation, and extracellular matrix accumulation perhaps through suppressing tumor necrosis factor and other cytokines.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Pathogenesis

MCD and FSGS are similar clinically and pathologically, and there is debate as to whether they are separate diseases or different ends of a single disease spectrum. Their origins are also, not surprisingly, thought to be similar and likely derive from an immunologic source. A role for T cells in the pathogenesis of MCD was first suspected in the 1970s, and some evidence has accumulated in recent decades to support this hypothesis. A T cell–derived soluble “permeability” factor, perhaps the Th-2 derived cytokine IL-13 (11), may impair the glomerular capillary wall or slit

diaphragm's ability to exclude larger proteins. Other molecules, including angiopoietin-4 (12), have been implicated as possible permeability factors.

Secondary Causes

Most cases of MCD are idiopathic; however, in a small population of patients a secondary cause can be found (Table 15-5). MCD has been associated with a number of drugs, malignancies, infections, environmental allergens, and other glomerular diseases.

Presentation

Adults typically present with mild renal impairment and the sudden onset of nephrotic syndrome, including proteinuria >3.5 g/24 hours hypoalbuminemia, hyperlipidemia, and edema. Hypertension is common. Microscopic hematuria is not uncommon. In a retrospective review, 20% of patients had acute kidney injury (AKI) at the time of presentation (13).

Prognosis

The prognosis of adult onset MCD is generally good and is related to how patients respond to the initial treatment with steroids (see section on "Treatment"). In current clinical practice most patients are treated at the time of diagnosis; however, there are some older data that suggest a spontaneous remission rate of anywhere from 20% to 65% (14,15). Adults tend to respond to therapy more slowly than children, often requiring over 3 months of therapy before remission is detected (13,15). Over 70% of treated adults have a complete remission (13), but the relapse rate is common with almost 60% to 75% of patients having at least one relapse and 30% to 40% of patients having frequent relapses. Patients who present with AKI can typically expect to return to their baseline renal function with treatment. Progression to end-stage renal disease (ESRD) is uncommon in MCD but is reported in steroid-resistant cases (15); however, subsequent biopsies may demonstrate FSGS that may be from progression of MCD or the original diagnosis that was missed by sampling error.

When children with MCD enter adulthood, less is known about their relapse rate and prognosis. One study found that among steroid-sensitive children, young age at onset (<6 years) and more severe disease in childhood, indicated by a greater number of relapses and more frequent

use of immunosuppressive drugs, were predictive of the occurrence of MCD relapse in adulthood when evaluated by univariate analysis. On multivariate analysis, only the number of relapses during childhood was predictive of adulthood relapses (16). Severe nephrotic syndrome has nonrenal-related complications as well, including thrombosis and infections (see Chapter 14). Complications arising from these disorders can also impact morbidity and mortality.

Treatment

There are no large, randomized controlled trials on treating adults with MCD. The majority of studies that have helped guide treatment are from the pediatric literature. Prednisone is the treatment of choice for adult MCD (17), with the majority of adults experiencing a complete remission by 3 months. Experts have different strategies for dosing prednisone, length of treatment, and tapering. Generally, prednisone is dosed at 1 mg/kg/d or 2 mg/kg every other day and continued for 2 months. If at 2 months complete remission has been obtained, prednisone is slowly tapered. If at 2 months a remission has not occurred, then the high-dose daily prednisone is continued. If a remission has not been achieved by 4 months, the patient is considered to be steroid resistant and other agents are required. Second-line agents typically used include cyclophosphamide, tacrolimus, cyclosporine, mycophenolate mofetil (MMF), and rituximab. Each agent has been shown to be useful in treating steroid-dependent and steroid-resistant patients; however, there are no adequately powered, randomized studies to help guide practitioners toward one treatment over another.

Relapses of MCD occur frequently. If patients responded well to prednisone during original treatment and there are no contraindications against more prednisone, then a shorter course of high-dose daily prednisone is usually tried. For frequent relapsers, low-dose prednisone over an extended period of time is a reasonable choice. Again, the second-line agents mentioned earlier have also been employed in treating frequently relapsing disease.

Table 15–5 Selected Causes of Secondary Minimal Change Disease

Neoplasia	Hodgkin disease, non-Hodgkin lymphoma, pancreatic carcinoma, bronchogenic carcinoma, allergic and dysregulated immune

	responses
Drugs	Nonsteroidal anti-inflammatory drugs, antibiotics, lithium, D-penicillamine

IgM NEPHROPATHY

IgM nephropathy is a rare disease that is characterized by the mesangial deposition of IgM and complement (18). Light microscopy may be normal or may show diffuse proliferation of mesangial cells and accumulation of mesangial matrix with varying degrees of sclerosis (18). EM usually shows dense deposits in the mesangium. Experts argue as to whether IgM nephropathy is its own entity, or whether it is a variant of MCD or FSGS and that the IgM deposits are a secondary event. Patients usually present with nephrotic syndrome, although patients with hematuria and asymptomatic proteinuria have been described (18). Patients with IgM nephropathy are less likely to respond to immunosuppressive agents when compared to patients with MCD (19).

C1q NEPHROPATHY

C1q nephropathy is another uncommon disorder that can be confused with MCD on light microscopy. By light microscopy, C1q nephropathy can present with no visible lesions, mesangial proliferation, FSGS, or a proliferative GN (20,21). Immunofluorescence demonstrates mesangial staining of C1q in all biopsies and mesangial IgG, IgM, and C3 in the majority of biopsies. IgA and C4 are not uncommon. EM can show mesangial, subendothelial, and subepithelial deposits with or without foot process effacement. Patients present with the nephrotic syndrome and hematuria. Renal function is usually normal but there have been reports of renal insufficiency at diagnosis. There is debate as to whether C1q nephropathy is its own disease or if it is a variant of MCD or FSGS. Most treatment strategies are based on the histologic lesion. Those with an FSGS pattern of injury are more likely to progress to ESRD.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

FSGS is one of the most common causes of the nephrotic syndrome in the United States and is the most common primary GN listed as the cause for ESRD (22,23). It shares many features with MCD and experts continue to debate whether the two processes are different diseases or whether FSGS

is just a more severe form of MCD. As with other glomerulonephritides, FSGS is a nonspecific pattern of injury diagnosed by renal biopsy. FSGS may be idiopathic or secondary. In order to make a diagnosis of idiopathic FSGS there must not be a history of any other GN, known systemic disease with possible glomerular involvement, or family history of FSGS (24). Secondary FSGS may be caused by structural functional adaptations mediated by elevated glomerular pressure, genetic diseases, or as the final common histology of other glomerulonephritides. Differentiating between primary and secondary FSGS is often clinically challenging.

FSGS has several morphologic variants that are seen with light microscopy. Generally, FSGS is characterized by the presence of some but not all glomeruli (focal) having segmental mesangial collapse and glomerular sclerosis with partial occlusion of the capillary loops by hyaline material. Hyalinosis, acellular material within the sclerotic area caused by the insudation of plasma proteins, used to be considered a specific histopathologic feature of FSGS but is now not a diagnostic necessity. Mild mesangial hypercellularity may be seen. Tubular atrophy and interstitial fibrosis are frequently present. EM shows foot process effacement, but in secondary forms of FSGS the extent of effacement is usually less than in primary disease (25,26).

Five histologic variants of FSGS have been described: classic FSGS, collapsing variant, tip variant, perihilar variant, and the cellular variant (27). The different lesions have different presentations and responses to therapy (see “Presentation” and “Prognosis” sections below). The collapsing variant shows collapse and sclerosis of the entire glomerular tuft with podocyte hypertrophy resembling pseudo-crescents (28). The tip variant shows a lesion adjacent to the “tip” of the glomerulus, which is the area next to the origin of the proximal tubule (27). The perihilar variant consists of hyalinosis and sclerosis adjacent to the hilum of the glomerulus. This lesion is frequently observed in secondary FSGS thought to be caused by increased capillary pressure at the hilum. The cellular variant shows segmental endocapillary hypercellularity that occludes the capillary lumen. The cellular lesion is very similar in appearance to the collapsing lesion and many pathologists make no distinction between the two variants. In a study examining the recurrence of FSGS in renal allografts, 81% of recurrences occurred in the same pattern as the original disease, giving credence to the different variants proposed (29).

Uninvolved glomeruli in FSGS show no lesions and one segmentally sclerosed glomerulus is sufficient to make the diagnosis of FSGS. Experts have concluded that it takes a biopsy containing 20 glomeruli to decrease

the risk of missing glomeruli affected by FSGS by sampling error to approximately 10% (30). FSGS usually starts in the corticomedullary glomeruli, so a biopsy should have adequate evaluation of this region.

Pathogenesis

The podocyte appears to be at the focal point of FSGS and injury to this resident renal cell likely initiates the process that leads to the FSGS pattern of injury. In idiopathic FSGS, as in MCD, a circulating permeability factor has been implicated as the cause of podocyte injury (31). This permeability factor has been blamed for the rapid onset of recurrent FSGS in newly transplanted kidneys. The soluble urokinase plasminogen activating receptor (suPAR) was identified as a circulating factor that might be related to FSGS (32), although this has not been confirmed in more recent studies (33).

Variants in the apolipoprotein L1 (*APOL1*) gene on chromosome 22 have also been associated with FSGS in African Americans (34). Polymorphisms in APOL1 are also only thus far seen expressed in those of African ancestry. A number of other genetic forms of FSGS have been identified and are discussed in other sections of this chapter.

Podocyte injury is also likely the inciting event in secondary FSGS and is caused by the compensatory hypertrophy of functional glomeruli that occurs after other glomeruli are lost or injured (35). This hypertrophy causes podocyte connections to the basement membrane and to other podocytes to become diminished; this leads to podocyte detachment and loss. Inflammatory cell infiltration, extracellular matrix accumulation, resident renal cell proliferation, and cytokines then contribute to the sclerotic scar (36).

Secondary Causes

FSGS can occur secondary to a number of different disorders. These can be broken down as structural or functional adaptations, genetic diseases, viral infections, drugs, or as a final pattern of injury after another GN (Table 15-6).

Presentation

FSGS can occur in all age groups. It affects a disproportionate number of African Americans. Patients with primary FSGS usually present with the

acute onset of the nephrotic syndrome—peripheral edema, hypoalbuminemia, and nephrotic range proteinuria. Hypertension is common. Renal function may be diminished at the time of diagnosis. Microhematuria is not uncommon. Secondary FSGS typically presents with nonnephrotic proteinuria, renal insufficiency, and hypertension.

Of the different variants, the tip and cellular/collapsing lesions frequently have a greater amount of proteinuria (37,38). The cellular/collapsing variants also usually have more severe renal dysfunction at presentation (37).

Table 15–6 Selected Causes of Secondary Focal Segmental Glomerulosclerosis

Viral	Human immunodeficiency virus, Parvovirus B19
Drugs	Pamidronate
Structural/functional adaptation to hyperfiltration or reduced renal mass	Reflux nephropathy, papillary necrosis, sickle cell disease, cholesterol embolization, unilateral renal agenesis, obesity, low birth weight
Other glomerular diseases	Minimal change disease, diabetic nephropathy, membranous nephropathy, healing phase of any inflammatory glomerular process

Prognosis

Untreated FSGS usually follows a progressive course leading to ESRD. Immunosuppressive treatment for idiopathic FSGS has a significant chance of improving outcomes if a partial or complete remission is obtained (39). Higher levels of proteinuria and increased serum creatinine at the time of diagnosis are predictive of lower renal survival. Histologic findings may also predict outcomes, and many experts believe that the “tip” variant is more responsive to therapy and thus more likely to have a favorable prognosis. The collapsing/ cellular variant portends a poor prognosis. As in other renal diseases, the amount of interstitial fibrosis predicts poor renal survival. Idiopathic FSGS can return in renal allografts.

Treatment

Only patients with idiopathic FSGS should be treated with immunosuppressive medications, and those patients with idiopathic FSGS and nephrotic range proteinuria are almost universally offered aggressive therapy, although discretion should be taken with those patients who have significant renal dysfunction. There are no large, randomized, placebo-controlled trials investigating the best initial treatment for idiopathic FSGS; however, most experts agree that an initial trial of prednisone (1 mg/kg) for at least 16 weeks followed by a taper based on patient response is the best initial strategy (40). Although the criteria can vary, a complete response is often defined as a reduction in proteinuria to <300 mg/day, and a partial response is often defined as at least a 50% reduction in proteinuria. Published reports indicate that close to half of those treated will have at least a partial response to therapy (39). In patients who cannot tolerate high-dose steroids, calcineurin inhibitors with low-dose prednisone have been shown to be effective, but the relapse rate is high.

Relapsing FSGS manifested by nephrotic range proteinuria after either a complete or partial remission is often treated with another round of steroids if the side effects were minimal during the first period of treatment. If steroid side effects were significant, cyclosporine or tacrolimus and low-dose prednisone are recommended. FSGS is considered steroid dependent if a patient relapses while on therapy. Steroid-resistant FSGS is defined by little or no reduction in proteinuria after 12 to 16 weeks of adequate prednisone therapy or if the criteria for partial remission are not met.

Experts recommend that both steroid-dependent and steroid-resistant FSGS be treated with calcineurin inhibitors combined with low-dose prednisone (40,41). Other agents have been used and reported on for refractory disease, including adrenocorticotropic hormone (ACTH), rituximab, and the co-stimulation blocker abatacept; however, the data are sparse and mainly consist of observational reports. For patients who have failed other therapies or who should not be exposed to calcineurin inhibitors, MMF may be effective (42). Plasmapheresis is also available for patients with primary FSGS after failure of other therapies.

Nonimmunosuppressive therapy with renin–angiotensin–aldosterone system (RAAS) inhibitors (e.g., angiotensin-converting enzyme [ACE] inhibitors or angiotensin receptor blockers [ARBs]) should be used in all patients with idiopathic or secondary FSGS. Blood pressure should be well controlled. Lipids should be managed with statin medications.

MEMBRANOUS NEPHROPATHY

MN is one of the most common causes of the nephrotic syndrome. MN is diagnosed by kidney biopsy. The characteristic histologic lesion on light microscopy is diffuse thickening of the GBM, with lesions usually affecting all glomeruli. The glomeruli may appear normal by light microscopy early in the course of the disease. Chronic sclerosing glomerular and tubulointerstitial changes develop as the disease progresses. Immunofluorescence shows a diffuse granular pattern of IgG and C3 staining along the GBM. EM demonstrates subepithelial electron-dense deposits, effacement of the podocyte foot processes, and expansion of the GBM. Advanced disease may have the presence of "spikes" of membrane interdigitating between immune deposits.

Making a distinction between idiopathic MN and secondary MN can be challenging. There are certain clues on biopsy that can be helpful. In idiopathic MN, electron-dense deposits on EM are almost exclusively subepithelial and intramembranous (43). Secondary forms of MN are often associated with mesangial and/or subendothelial deposits, which suggest a circulating IC (43). Tubular basement membrane staining for IgG on immunofluorescence is rare in idiopathic MN, but is common in secondary forms such as SLE (43).

Pathogenesis

MN is an IC-mediated disease as evidenced by the presence of subepithelial ICs visualized by immunofluorescence and EM. Several podocyte antigens have been identified in subsets of patients with idiopathic MN. In the majority of patients, antibodies specific for the phospholipase A_2 receptor are detectable in the serum and in the glomerular deposits (4). Antibodies to thrombospondin type-1 domain-containing 7A (THSD7A) are also seen in some patients with MN (44). Interestingly, MN patients with antibodies to THSD7A do not have antibodies to the phospholipase A_2 receptor, indicating that autoimmunity to these proteins occurs in distinct groups of patients but presents with a similar clinical phenotype. Cases have also been reported in which neutral endopeptidase (NEP), an enzyme present on podocyte cell surfaces, has acted as a target antigen for anti-NEP antibodies that crossed the placenta and caused MN in infants (45).

Antibodies to the phospholipase A_2 receptor are usually of the IgG4 subclass, which is a poor activator of the complement system, yet glomerular C3 deposits are seen in the majority of patients (46). It is not

yet certain how the deposited antibody activates the complement cascade. Once activated, however, the complement system causes podocyte injury via the sublytic action of the membrane attack complex (C5b–9) inserted into podocyte membranes.

Secondary Causes

Secondary causes of MN represent 25% to 35% of all patients, with a slightly higher prevalence in children and older adults (Table 15-7) (47). In 85% of secondary causes, the etiology can be attributed to infections, neoplasia, or lupus (47). Infections that have been reported to cause MN include hepatitis B, hepatitis C, human immunodeficiency virus (HIV), syphilis, schistosomiasis, and malaria (43). A minority of older adults with MN have an associated cancer, usually a solid tumor and less frequently a hematologic malignancy (43). The list of medications and toxic agents associated with MN is long and includes penicillamine, gold salts, nonsteroidal anti-inflammatory drugs (NSAIDs), captopril, anti-tumor necrosis factor (TNF) agents, mercury, and formaldehyde (43). The mechanisms responsible for drug-induced MN are uncertain. Hematopoietic cell transplantation (HCT) and graft-versus-host disease can also cause MN. De novo MN can occur in transplanted kidneys. In addition, there are a number of case reports linking several other disease states to MN, including sarcoidosis and other autoimmune diseases like Sjögren syndrome and thyroid disease (43).

Presentation

MN affects patients of all ages but has a peak incidence in the fourth to fifth decade. All ethnic groups can be affected. The diagnosis is made in more men than women in a 2:1 ratio (48). Almost 70% of patients present with the nephrotic syndrome as evidenced by severe proteinuria, low albumin, and edema (49). Lipid abnormalities are common. The rest of the patients present with subnephrotic range or asymptomatic proteinuria. The onset of clinical symptoms is usually gradual perhaps matching the rate of membranous deposit formation. Microscopic hematuria is common but macroscopic hematuria and red blood cell casts are rare (49). Renal function is usually normal at diagnosis. Only a minority of patients have hypertension at the time of diagnosis. Serum complement levels are usually normal. In patients with secondary MN, other clinical or laboratory findings may be present that are attributable to the primary disease

process. In patients with an underlying malignancy, almost half had a known cancer diagnosis at the time of renal biopsy, whereas the rest had a MN diagnosis before any cancer diagnosis (50). Work up of an adult patient with MN should include age-appropriate cancer screens or a direct evaluation of symptoms if present. MN has also been well documented to occur concurrently with other glomerular diseases, including diabetes, IgA, FSGS, and crescentic GN.

Table 15–7 Selected Causes of Secondary Membranous Disease

Neoplasia	Lung, colon, breast, stomach, bladder, thyroid, prostate, pancreas, kidney, malignant melanoma, Hodgkin disease, non-Hodgkin lymphoma, CLL
Infection	Hepatitis B, hepatitis C, HIV, malaria, schistosomiasis, syphilis, filariasis
Autoimmune disease	Lupus, rheumatoid arthritis, mixed connective tissue disease, Sjögren disease
Drugs	NSAIDs, gold, penicillamine, captopril, probenecid, clopidogrel, anti-TNF agents
Other systemic disease	Sarcoidosis, sickle cell disease, hematopoietic stem cell transplantation
Other renal diseases	Polycystic kidney disease

CLL, chronic lymphocytic leukemia; HIV, human immunodeficiency virus; NSAID, nonsteroidal anti-inflammatory drug; TNF, tumor necrosis factor.

Prognosis

Spontaneous complete or partial remission of proteinuria occurs in approximately 50% to 60% of patients within 5 years. The remainder of untreated patients develop progressive renal insufficiency over the next 15 years (51,52). Clinical predictors that signal an increased risk of progressive renal decline include age >50 years, male sex, protein excretion >8 g/24 hours, and an increased serum creatinine at presentation (53,54). Histologic findings that may be associated with risk of progression are glomerular scarring and the severity of the tubulointerstitial disease at the time of biopsy (55). Given the toxicity of available medications used to treat MN and the difficulty in establishing what patient group to treat, the Toronto Glomerulonephritis Registry established a model to help classify patients by risk (54). Patients who

present with normal renal function, proteinuria <4 g/24 hours, and stable renal function over a 6-month observation period have excellent long-term prognosis and are considered low risk. Patients with normal renal function at diagnosis that remains stable over 6 months but who have between 4 and 8 g/24 hours of proteinuria are considered medium risk and have a 55% chance of developing progressive renal insufficiency. Patients with persistent proteinuria >8 g/24 hours, independent of renal function, have close to an 80% chance of progressing and are considered high risk. Patients who were never nephrotic or who obtain a complete remission of proteinuria have good long-term renal survival, and a partial remission also predicts improved long-term outcome (56).

Treatment

All patients with MN should be on an RAAS inhibitor for both blood pressure and proteinuria control. Lipids should be controlled. As described above, the decision to initiate immunosuppressive therapy treatment for MN should be based on the patient's risk for progressive renal decline. Low-risk patients should have continual monitoring but most experts do not recommend disease-specific therapy. Moderate-risk patients who have not improved their degree of proteinuria to <4 g/24 hours over the observation period should be started on immunosuppressive therapy. Available options include a cyclophosphamide/steroid regimen, calcineurin/steroid-based protocols, and rituximab (51,57–59). The cyclophosphamide/steroid regimen typically consists of 6 months of therapy. Steroids are administered in months 1, 3, and 5, and cyclophosphamide is administered in months 2, 4, and 6. If patients do not respond to the initial therapy, most experts then recommend trying one of the alternative regimens. Synthetic ACTH may be effective in some patients who do not respond to conventional treatments (60). Studies using MMF have yielded varying results (61,62).

High-risk patients are initiated on treatment if there is no improvement in protein excretion after only 3 months or if renal function is reduced and thought secondary to MN.

Relapse of MN, as evidenced by an increase in proteinuria after a remission, occurs in approximately 30% of patients treated with cyclophosphamide-based protocols and 40% of those treated with calcineurin inhibitors. In patients whose antibodies to the phospholipase A_2 receptor decreased after treatment, an increase in the titer of these antibodies may predict a relapse (63). There is not a consensus on how to

treat relapses, but the decision is generally between re-treating the patient with the original protocol versus attempting the other protocol. Decisions are based on how well the patient tolerated the first round and if other side effects can be minimized.

In patients with secondary MN, cessation of the offending drug or effective treatment of the underlying disease is usually associated with improvement in the nephrotic syndrome.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

MPGN is defined by its morphologic characteristics on renal biopsy. Typically, there is mesangial expansion and hypercellularity, causing a lobular appearance of the glomerular tuft (64,65). Mesangial interposition into the walls of the capillaries causes the GBM to split, causing reduplication of the GBM and a "double contour" best seen with silver–methenamine–period acid–Schiff stains. Mesangial interposition into the capillary wall and subendothelial IC deposits cause thickening of the capillary wall. C3 deposits in the capillary loops are virtually always seen by immunofluorescence microscopy, and granular deposits of IgG are also usually seen.

Type I MPGN is a rare form of primary GN, and this pattern of glomerular injury may also be caused by IC deposition in patients with chronic infections, cryoglobulinemia, autoimmune disease, malignancy, and sickle cell disease (Table 15-5). This morphologic pattern of injury has also been termed mesangiocapillary GN (66). MPGN typically occurs in children or young adults and accounts for approximately 5% of GN. MPGN type II, or dense deposit disease (DDD), is a histologic variant of MPGN that has pathognomonic electron-dense deposits in the GBM (67). Even though DDD can have a MPGN pattern of injury on light microscopy, it is now classified as a form of C3G and will be discussed in a subsequent section of this chapter. A pattern of glomerular injury that is similar to MPGN type I but with abundant subepithelial IC deposits and rupture of the GBM has been termed "MPGN Type III" (68,69).

Pathogenesis

Circulating ICs are commonly detected in patients with MPGN (70). IC deposition in the subendothelial space and in the mesangium is probably critical to the development of both the primary and secondary forms of MPGN type I. The ICs activate complement and immunoglobulin

receptors (Fc receptors) triggering the recruitment of neutrophils and monocytes. Activated inflammatory cells release reactive oxygen species and proteases. These factors and complement activation fragments can directly damage the capillary wall. Platelets may also accumulate and contribute to glomerular injury by releasing chemotactic factors and growth factors. Patients with MPGN often have low platelet levels and a shortened platelet lifespan, supporting a role of platelets in this disease (71). Cytokines and growth factors may induce proliferation of mesangial cells and the production of mesangial matrix.

Patients with MPGN type I are frequently hypocomplementemic (72). This may reflect consumption of classical pathway components (e.g., C3 and C4) by the ICs. An autosomal dominant pattern of transmission of MPGN has been identified in one family and the disease was linked with the area on chromosome 1q, which contains the genes for the complement regulatory proteins (73).

Table 15–8 Selected Causes of Secondary Membranoproliferative Glomerulonephritis

Autoimmune disease	Lupus, Sjögren syndrome, rheumatoid arthritis, genetic mutations in complement regulatory proteins
Neoplasia	Plasma cell dyscrasias, leukemia, lymphoma, melanoma
Infection	Chronic bacterial infections, hepatitis C, hepatitis B, HIV, Coxsackie virus, Epstein–Barr virus

HIV, human immunodeficiency virus.

It seems likely that the mechanisms of glomerular injury in primary and secondary MPGN are similar, but that the antigens are distinct. The identity of the antigen in idiopathic MPGN is not known. The incidence of idiopathic MPGN type I has been decreasing in recent decades (74). It is possible that part of the decline of idiopathic MPGN is due to improved diagnosis of secondary causes, such as MPGN caused by hepatitis C and cryoglobulins. Future studies may identify new infectious causes for what is currently considered idiopathic disease.

Secondary Factors

As described above, a pattern of injury on light microscopy similar to idiopathic MPGN may be seen in patients with infectious, autoimmune,

and malignant disorders (Table 15-8). Many cases that once would have been regarded as “idiopathic” MPGN are now recognized as being caused by hepatitis C, and many other systemic diseases are associated with this pattern of glomerular injury. In most of these diseases, the glomerular injury results from persistent IC formation. This may be caused by infections that persist in spite of a humoral immune response, autoimmune diseases in which antibodies to endogenous antigens form ICs, or hematologic malignancies.

Presentation

Patients with MPGN can present with a nephritic syndrome, nephrotic syndrome, and sometimes with an RPGN. Patients presenting with nephritic features usually have microscopic hematuria, but episodes of macroscopic hematuria can occur. Idiopathic MPGN is generally a renal limited disease, but patients often have fatigue, anorexia, and weight loss. Hypertension and anemia are also seen in many patients. Complement levels are frequently depressed in patients with MPGN type I. C3 levels are low in approximately 70% of the patients, and C4 levels are also frequently low.

Prognosis

The course and natural history of untreated MPGN are variable. A minority of patients may have spontaneous remissions, but most have persistent proteinuria. The renal function can slowly decline, and there may also be periods of rapid deterioration. Untreated, renal survival is approximately 50% at 10 years (75). Because of the variable course of MPGN type I and the small size of clinical trials, it is difficult to assess whether treatment of the disease significantly improves the outcome. Uncontrolled studies in children, however, suggest that treatment may improve long-term outcomes, with 10-year renal survival reaching 75% (76). Factors that may predict a worse prognosis include nephrotic range proteinuria, tubulointerstitial fibrosis on biopsy, and renal dysfunction 1 year after diagnosis (77).

Treatment

Patients should receive nonspecific therapies such as control of the blood pressure, and RAAS inhibitors should be used in patients with proteinuria.

Complications of the nephrotic syndrome should also be treated. Uncontrolled studies in children suggest that treatment with corticosteroids may improve the outcome of MPGN type I, although not all authors agree that the risks of treatment are justified given the limited efficacy data. Patients can be treated with 40 mg/m^2 of prednisone on alternate days. In patients with severe disease, the high dose can be maintained for 2 years, and then tapered to 20 mg/m^2 and maintained for prolonged periods. There is less evidence for the efficacy of prednisone in adults, but in patients with severe disease, a 6-month course of prednisone (1 mg/kg/d) can be tried. Antiplatelet agents, such as aspirin and dipyridamole, may be of benefit (78). There are no controlled trials to support the use of cytotoxic agents in patients with MPGN. There are several reported cases of steroid-resistant MPGN that were successfully treated with mycophenolate, but no randomized trials of this agent have been performed.

C3 GLOMERULOPATHY

DDD refers to glomerular disease associated with electron-dense deposits in the GBM or electron-dense transformation of the GBM (79). As mentioned above, DDD was initially regarded as a variant of MPGN and was referred to as MPGN type II, but kidneys that demonstrate dense deposits by EM (the pathognomonic feature of DDD) do not always have an MPGN pattern by light microscopy (79). Immunofluorescence microscopy of DDD kidneys typically shows abundant C3, but not IgG or C4. The classification of these glomerular diseases was further modified when it was discovered that some biopsies have dominant C3 deposits by immunofluorescence but do not have the characteristic dense deposits by EM (1). Renal biopsies with dominant C3 deposits (at least two orders of magnitude greater than other immune deposits) are now termed as having C3G (80). If dense deposits are seen in the GBM by EM, the disease falls in the subcategory of DDD, and the term C3 glomerulonephritis is used for kidneys in which the characteristic dense deposits are not seen.

There is compelling genetic and animal data demonstrating that unregulated activation of the alternative pathway of complement is central to the development of C3G (81). C3 nephritic factor (C3NeF) is an autoantibody that stabilizes the alternative complement pathway C3 convertase, thus amplifying complement activation through this pathway. Greater than 85% of patients with DDD have detectable C3NeF, and >80% of DDD patients with active disease have decreased levels of circulating C3. In some patients, mutations in factor H (a circulating protein that

regulates the alternative pathway) are associated with disease, and a number of other mutations and autoantibodies that cause dysregulated activation of the alternative pathway have been identified (81–83). The diagnosis of C3G is based upon the immunofluorescence findings, not a particular pattern of injury by light microscopy. Immunoglobulin can be seen within the glomeruli, but currently the diagnosis of C3G requires that staining for C3 is the dominant finding (84).

DDD currently has a very poor prognosis with >50% of patients progressing to ESRD within 10 years of their diagnosis (85), although the prognosis may be slightly better for C3 glomerulonephritis (82). Blood pressure should be controlled and patients should be treated with RAAS inhibitors, but there are no specific treatments of proven benefit. Plasma exchange may be beneficial in patients with mutations in circulating complement regulatory proteins or who are positive for C3NeF (86). Eculizumab may be beneficial in some patients, but overall the results are mixed (87). Retrospective data also suggest that MMF may be beneficial in some patients (88).

IgA NEPHROPATHY

IgAN is an IC disease that is the most common form of GN in the world. In the United States and Western Europe, IgA accounts for 10% to 30% of GN, whereas in China, Japan, and Korea, IgA causes close to 50% of GNs (89,90).

IgAN is diagnosed by renal biopsy. There have been attempts to use serum and urine markers to predict a diagnosis of IgA, including the serum IgA/C3 ratio (91), urine proteomics (92), and serum galactose-deficient IgA levels (93); however, no large-scale studies have been performed to determine if these are sufficiently sensitive and/or specific to reliably aid in making the diagnosis of IgAN. IgAN can have any of the histologic phenotypes of IC-mediated GN, including no lesion by light microscopy, mesangioproliferative GN, proliferative GN, and crescentic GN. A classification system known as the Oxford classification of IgAN, developed by the International IgA Nephropathy Network in collaboration with the Renal Pathology Society, is similar to the World Health Organization’s (WHO) system for lupus (94). Pathology reports should provide numerical scores based upon the presence or absence of mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity, and tubular atrophy/interstitial fibrosis, as these findings seem to correlate with patient outcomes.

The traditional light microscopy histology for IgAN is mesangial proliferation and matrix expansion (95). Crescents are not uncommon but full-blown crescentic GN is the exception. Immunofluorescence shows staining for IgA (IgA1 predominantly) in the mesangium often accompanied by low intensity staining for IgG and IgM. C3 staining is typically present as well. EM shows IC deposition in the mesangium.

IgA deposits have been documented in asymptomatic individuals, primarily in transplant studies (96). The significance of this is unknown. Also, IgA deposits have been reported in other forms of GN, including thin basement membrane nephropathy (TBMN), lupus nephritis, MCD, vasculitis, and DN. It is possible that these findings are nonspecific and unrelated to the primary disease; however, the true significance and clinical applicability remain largely unknown.

Henoch–Schönlein purpura (HSP) is a systemic vasculitis characterized by the deposition of IgA-containing ICs. It usually presents with a nonthrombocytopenic palpable rash (leukocytoclastic vasculitis), polyarthralgias, abdominal pain, and renal disease. The renal lesion of HSP is indistinguishable from IgAN and the pathogenesis is likely similar to IgAN as well. HSP occurs more often in children than in adults; however, adults and older children are likely to have more severe renal disease (97). HSP resolves spontaneously in the majority of adults and children. However, severe or persistent renal dysfunction often requires specific therapy (see section on “Treatment” below).

Pathogenesis

The recurrence of IgAN in transplanted kidneys implicates a circulating pool of IgA as opposed to local IgA production. Increased polymeric IgA1 plasma cells are found in the bone marrow and tonsils in IgAN (98,99). A number of studies have shown that patients with IgAN have increased levels of serum IgA, but that increased total levels of IgA are insufficient to cause IgAN. The IgA in patients with IgAN is anionic, overrepresented by λ-light chains, and has an *O*-glycosylation abnormality with reduced galactosylation of the IgA1 hinge region *O*-glycans, leading to increased frequency of truncated *O*-glycans (100). The changes in *O*-glycosylation only seem to become apparent after antigen encounter and may be linked to B-cell maturation and class switching to IgA1 synthesis. The IgA molecule produced in patients with IgAN has low affinity for its antigen, may be poorly cleared, and persists longer in circulation. Aberrantly galactosylated IgA1 molecules also have an increased tendency to self-

aggregate and form antigen–antibody complexes. The aberrant glycosylation may also serve as an antigen for autoantibody IgG, and the IgG-IgA1 forms ICs. These complexes are prone to mesangial deposition because of an altered number of sialic acid and galactose units secondary to aberrant *O*-glycosylation allowing binding to extracellular matrix fibronectin and type IV collagen. Genome-wide association studies have identified risk loci within the major histocompatibility complex (MHC), supporting an immune basis for this disease (101). Variants in the genes for the complement factor H–related proteins were also identified as risk factors, highlighting the importance of complement activation in this disease. IgAN is not usually associated with a cellular infiltrate, except in severe or crescentic disease, suggesting that the mesangial cells and complement mediate injury. Mesangial cells undergo a phenotypic transformation to a pro-inflammatory and pro-fibrotic cell upregulating secretion of extracellular matrix components, transforming growth factor (TGF)-β, platelet-activating factor, IL-1β, IL-6, and other cytokines and chemokines. IgA can activate both the mannose-binding lectin and alternative pathways of the complement cascade (102). This ultimately leads to the generation of the membrane attack complex causing further damage.

Secondary Causes

Idiopathic IgAN, including HSP, is far more common than secondary disease. The deposition of IgA has also been associated with a number of other clinical conditions, including cirrhosis, HIV, celiac disease, seronegative arthritis, and malignancies. Most adult patients with IgA deposition in association with other diagnosis are asymptomatic with relatively bland urine findings, except for HIV-associated IgAN, which can present more typically.

Presentation

IgAN can present at any age but typically does so in young adulthood. There is a male predominance with Caucasians and Asians being affected much more commonly than blacks. Patients with IgAN typically present in one of three ways (103). Almost half of the patients will present with one or more episodes of gross hematuria often times following an upper respiratory tract infection. These patients may have flank pain during acute episodes reflecting kidney edema and stretching of the renal capsule. Low-

grade fever may also be present. Another 30% to 40% of patients will have microscopic hematuria and usually mild proteinuria, which is detected on a routine examination. Gross hematuria may eventually occur in some of these patients. Less than 10% of patients present with either nephrotic syndrome or acute RPGN. It is thought that these patients have had undetected disease for some time.

Rarely, patients present with acute renal failure caused by either crescentic IgAN or by heavy glomerular hematuria leading to tubular occlusion and/or damage by red cells. The latter is usually reversible, although incomplete recovery of renal function has been described (104).

Prognosis

IgAN was initially considered to be a relatively benign disease. Now, with more insight into the disease and with more patient-years of follow-up, it has been determined that approximately half of all patients with IgAN will slowly progress to ESRD (105). Patients with >0.5 g/24 hours of proteinuria, elevated serum creatinine at diagnosis, and hypertension are at greatest risk for progression in the long term, but risk for progression exists in all manifestations of the disease (106,107). As described above, different classification schemes have been devised to describe the histology of IgAN; however, the degree of glomerulosclerosis and tubulointerstitial disease seem to be most strongly associated with a poor prognosis (107). The presence of crescents is also associated with a risk of progressive disease. Elevated serum levels of poorly galactosylated IgA1 may correlate with a higher likelihood of developing ESRD (108). There is also evidence that C4d staining within the mesangium indicates a worse prognosis (109). Histologic recurrence with or without clinical disease can occur in transplanted kidneys.

Treatment

Treatment of IgAN is typically based on disease severity; however, there is a lack of any unified algorithm that is widely accepted. Part of this uncertainty results from the slow progression of IgAN, which is further complicated by the patient-specific variability of disease progression.

Most experts agree that patients with isolated hematuria, minimal proteinuria, and normal renal function need do not need specific treatment other than possibly initiating treatment with RAAS inhibitors. For patients with >0.5 g/24 hours of proteinuria, a RAAS inhibitor titrated to a dose

that attempts to normalize proteinuria is recommended. Evidence suggests that even obtaining a partial remission of proteinuria dramatically slows down progression of renal function decline (110). There have been both positive and negative studies regarding the use of high-dose fish oil (ω-3-polyunsaturated fatty acids) supplements and their ability to slow down the decrease in GFR. If tolerated, some experts advocate using them when RAAS inhibitors are added.

For patients with progressive disease manifested by increasing serum creatinine or proteinuria, a trial of steroids can be tried with the goal of reducing proteinuria and improving renal survival, although the results of trials have been mixed (111–114). Regiments including pulse methylprednisolone with oral prednisone or only oral prednisone are described.

For patients who fail to respond to steroid therapy alone or have severe disease at presentation, daily oral cyclophosphamide and steroids with or without azathioprine are typically initiated to decrease proteinuria and improve long-term renal function (115). For patients with crescentic GN and a rapidly progressive clinical course, therapy with intravenous pulse glucocorticoids and cyclophosphamide is recommended. Some experts also advocate the use of mycophenolate or azathioprine (116,117). Other more experimental therapies include tonsillectomy and low antigen diets.

Glomerulopathies Associated with Multisystem Disease

INFECTION-RELATED GLOMERULONEPHRITIS

Poststreptococcal GN (PSGN) is a syndrome primarily seen in children in which acute GN develops 1 to 4 weeks after a streptococcal infection. Other bacterial infections are associated with the development of GN concurrent with the infection, particularly if the infection is chronic. Well-described causes include bacterial endocarditis (both subacute and acute cases), chronically infected ventricular shunts, abscesses, and bacterial pneumonia (118). The epidemiology of GN associated with endocarditis has changed in recent years. Improved prevention and treatment of subacute bacterial endocarditis caused by organisms such as *Streptococcus viridans* has reduced the incidence of GN associated with this disease, whereas GN caused by acute endocarditis with *Staphylococcus aureus* has risen.

Presentation

PSGN typically presents as an acute nephritic syndrome that starts within several weeks of an infection with β-hemolytic streptococci. There is typically a latent period between the resolution of the infection and the onset of GN (119). Most cases of PSGN are caused by group A streptococci, and renal disease can develop after pharyngitis or after streptococcal infections of the skin. Cases of PSGN can occur sporadically or they can occur in epidemics. In either instance, it occurs more commonly in children than in adults, and males are affected slightly more commonly than females. The triggering infection in patients with PSGN is not always clinically evident. Patients with PSGN usually present with edema, hematuria, and proteinuria (usually subnephrotic). The urinary sediment is almost always "active," and dysmorphic red blood cells may suggest a glomerular etiology. Patients can develop renal failure, some with oliguria. Rapidly progressive renal failure with prominent crescents on biopsy can also occur, but only in a small percentage of patients. Hypertension is common. Patients may develop seizures secondary to the hypertension, but there is evidence that this may also be caused by cerebral vasculitis (120).

More than 90% of patients with active PSGN have a low C3 level (121). The C4 is generally normal, consistent with activation through the alternative pathway. Serologic tests may demonstrate antibodies reactive with streptolysin (ASO), hyaluronidase (AHase), streptokinase (ASKase), nicotinamide-adenine dinucleotidase (anti-NAD), and DNAse B (122), but these antibodies may not be detectable in patients who have received antibiotics.

On biopsy, PSGN usually causes endocapillary and mesangial proliferation. The lesion is often termed "exudative" because of the presence of abundant PMNs in the glomeruli. Over time, fewer numbers of PMNs are seen but the glomeruli may contain mononuclear cells. Glomeruli may also contain fibrin thrombi and areas of necrosis. Immunofluorescence microscopy often reveals IgG or IgM. C3 is invariably present in the mesangium and in the capillary walls. This can cause a "starry-sky" pattern of fine, scattered deposits, or a "garland" pattern of large deposits in the glomerular tuft (123). Large subepithelial electron-dense "humps" are classic for PSGN (Fig. 15-5), and mesangial and subendothelial deposits may also be seen.

Patients with GN associated with active bacterial infections usually develop hematuria and proteinuria. If the glomerular involvement is

diffuse, patients may develop the nephrotic syndrome, gross hematuria, or renal insufficiency. Systemic symptoms commonly include fever, purpura, and arthralgias. In most instances, the renal injury is caused by glomerular ICs. The location of the ICs may determine the histologic appearance and the clinical presentation. A proliferative pattern is usually seen by light microscopy, and glomerular ICs are accompanied by C3 deposits by immunofluorescence. IgA is the dominant immune deposit in some patients with GN caused by staphylococcal infections (124). Systemic C3 levels are usually depressed during the acute illness, although C3 levels are typically normal in patients with abdominal abscesses or non-endovascular infections with methicillin-resistant *Staphylococcus aureus*. Antineutrophil cytoplasmic antibodies (ANCA) can be present in elderly patients and in up to 25% of patients with endocarditis (118). Circulating and deposited cryoglobulins are also frequently present in patients with infection-associated GN. In early series, chronic bacterial or tubercular infections caused a significant percentage of the cases of AA amyloidosis, but a more recent series indicates that infection is now rarely the cause (125).

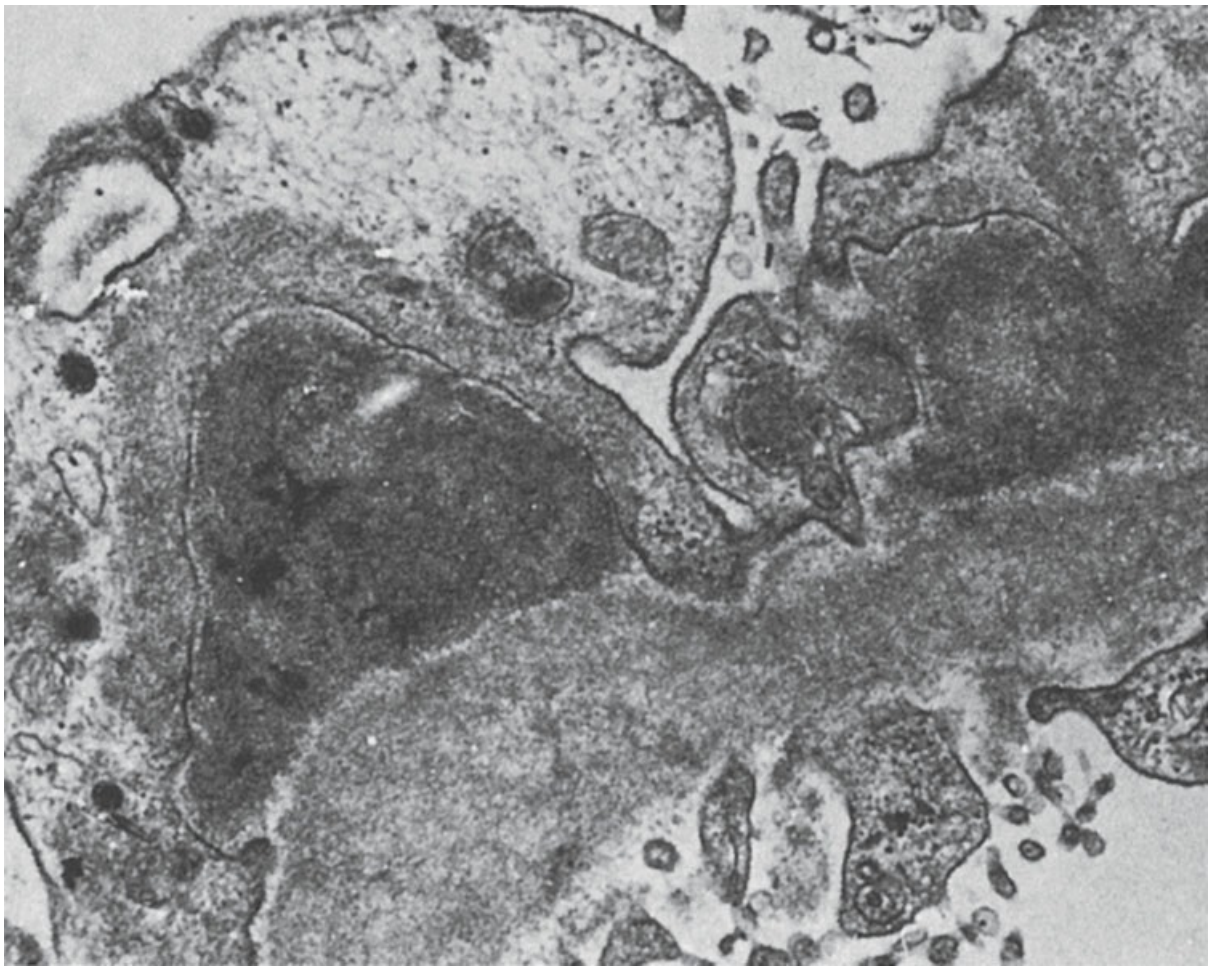

Figure 15–5 Subepithelial deposition of electron-dense material (humps), which may

be found in several types of IC glomerulopathies and is characteristic of poststreptococcal diffuse endocapillary proliferative glomerulonephritis.

Pathogenesis

Two streptococcal molecules may be pathogenic in PSGN: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and streptococcal pyrogenic exotoxin B (126). These molecules may bind within the glomerulus and directly activate the alternative pathway of complement. They may also provide the antigen for ICs that deposit or form in the capillary wall. Circulating cryoglobulins and rheumatoid factor are also common, suggesting that these antibodies may contribute to the glomerular injury. GN associated with other bacterial infections is thought to be mediated by glomerular ICs. These may form *in situ* after bacterial antigens are planted within the glomerular capillary or they may be caused by the deposition of circulating ICs.

Prognosis and Treatment

Even in patients with severe renal dysfunction, PSGN is usually self-limited and renal function returns to normal within a few weeks. Proteinuria and hematuria can however persist for months or even years. The “garland” pattern on biopsy is associated with a greater likelihood of persistent proteinuria. PSGN only rarely causes ESRD, but some patients go on to develop hypertension or chronic renal disease. Adults are probably more likely than children to develop chronic renal disease. ACE inhibitors may be beneficial, but there are no studies to support improved outcomes with corticosteroids or immunosuppressive medications. There are case reports of patients with severe, crescentic disease who appeared to benefit from treatment with corticosteroids, but controlled trials are lacking.

The clinical presentation of GN associated with active infections is similar to that of PSGN, although the degree of renal failure is more severe in elderly patients. Mildly impaired renal function usually improves with treatment of the underlying infection. Patients with crescentic disease or severe renal impairment, however, may have a progressive decline in renal function in spite of antibiotic therapy. Immunosuppressive drugs and plasmapheresis are of uncertain benefit, and should not be used unless the underlying infection has clearly been eradicated.

HIV-RELATED GLOMERULAR DISEASE

HIV-infected patients make up at least 2% of the population with ESRD (127). The prevalence of renal disease related to HIV infection before the highly active antiretroviral therapy (HAART) era is largely unknown; however, a small European autopsy study of primarily Caucasian patients with AIDS found renal pathology in 43% (128). A number of studies in the HAART era have estimated that close to 20% of HIV-infected patients have chronic kidney disease (CKD) (129,130). Patients of African descent with HIV are at particularly high risk of progression to ESRD, and the risk is increased in patients carrying the APOL1 risk allele (131).

There are three main classes of HIV glomerulopathies, including HIV-associated nephropathy (HIVAN), HIV immune–mediated GN, and thrombotic microangiopathy secondary to HIV (132). HIVAN and HIV immune–mediated GNs will be discussed here. A discussion of thrombotic microangiopathy can be found elsewhere in this chapter. Other complications of HIV/AIDS including drug-associated renal injury, neoplasms, metabolic abnormalities, and AKI secondary to opportunistic infections can also contribute to renal morbidity and can be found elsewhere in this book.

HIVAN

The characteristic histologic findings for HIVAN by light microscopy include collapsing FSGS, podocyte hypertrophy, tubular epithelial atrophy with microcystic dilatation of the tubules, and lymphocytic infiltration. Immunofluorescence is usually nonspecific. EM may show endothelial tubuloreticular inclusions related to high plasma interferon (IFN) levels.

Pathogenesis

The exact pathogenesis of HIVAN is unknown, but it is likely caused by viral infection of resident kidney cells, including glomerular endothelial, epithelial, mesangial, and tubular cells. The presence of HIV may also stimulate the release of cytokines, including fibroblast growth factor and TGF-β, which then contribute to the matrix accumulation, fibrosis, and tubular injury seen in HIVAN (133,134). HIV gene products can directly induce cell-cycle progression, resulting in epithelial cell dedifferentiation and collapse (135).

The increased prevalence of HIVAN in African Americans implies

genetic factor involvement as well. Studies have identified single nucleotide polymorphisms, present only in African Americans, in the APOL1 gene on chromosome 22 that are strongly linked to increased risk of HIVAN (131).The pathogenic role of APOL1 in HIVAN pathogenesis is not known (136).

Presentation

HIVAN can be present in any race but it is typically a disease of patients of African descent. HIVAN classically presents late in the course of HIV/AIDS with low CD4 counts and elevated viral loads. It can, however, also present anytime during the course of HIV with adequate CD4 counts and undetectable viral loads. Patients with HIVAN typically present with heavy proteinuria, hypoalbuminemia, renal dysfunction, and occasionally edema. Microscopic hematuria may also be present. The kidneys often are enlarged and echogenic on ultrasound examination corresponding to the lymphocytic infiltration and tubular dilation seen on biopsy. Blood pressure, surprisingly, is usually normal perhaps related to salt wasting that has been reported in HIVAN. In the HAART era, HIVAN has been reported to present with less severe findings.

Prognosis

When HIVAN was first described the prognosis was dismal, with patients progressing to ESRD in months. In the HAART era, the rate of progression of HIVAN to ESRD has been reduced by an estimated 40% (137). Some evidence also suggests that HAART may prevent the development of HIVAN (138).

Treatment

HAART is recommended as first-line therapy to treat HIVAN, even though there are only retrospective and nonrandomized trials demonstrating its effectiveness (139). RAAS inhibitors have been shown in small studies to delay the progression to ESRD and decrease proteinuria and should be used and titrated as tolerated (140). Steroids and cyclosporine have also been used in those patients not responding to HAART or RAAS inhibitor therapy (141). Survival rates of patients with HIV on dialysis are similar to non-HIV patients. Transplantation is also an option.

HIV Immune–Mediated Glomerulonephritis

Autopsy and biopsy studies have demonstrated a wide prevalence of immune-mediated GN in HIV-infected patients, with anywhere from 10% to 80% being reported (142). IC GN associated with HIV may present with proliferative, lupus-like (immunofluorescence positive for C1q, IgG, IgM, IgA, C3, λ, and κ), mixed proliferative, or sclerotic histology. Membranoproliferative, IgA, membranous, fibrillary, immunotactoid, and postinfectious GN have also been reported (143). The presence of hepatitis C or B coinfection must also be considered, as they are known to cause a number of the histologic patterns of injury mentioned earlier.

Pathogenesis

Infection with HIV may lead to polyclonal hypergammaglobulinemia and to the development of circulating ICs, many of which are composed of HIV peptides and their associated antibody (144). Passive trapping of these complexes versus in situ formation of antigen–antibody complexes on glomerular cells can initiate the immune response and trigger IC GN.

Presentation

HIV immune-mediated GNs occur more frequently in Caucasians and Asians. Patients usually present with hypertension, an active urine sediment, proteinuria, and renal insufficiency. Low levels of serum complement proteins may be seen. Antinuclear antibody (ANA) and antibodies to double-stranded DNA (anti-dsDNA) are typically negative even in those patients with lupus-like histology. CD4 counts and HIV viral loads do not seem to be predictive of disease type or severity.

Prognosis

The prognosis of HIV immune–mediated GNs is largely unknown but is considered to be generally poor in most literature reports. Some studies have demonstrated that renal survival may be predicted in part by the degree of fibrosis at biopsy and by the amount of proteinuria.

Treatment

Little is known about the specific treatments for HIV immune–mediated

GNs. Most patients should likely be on an RAAS inhibitor for proteinuria reduction. The data are mixed about whether HAART improves proteinuria or delays progression of disease (145,146). There are case reports involving the use of immunosuppressant drugs with some degree of success (147,148).

HEPATITIS C VIRUS INFECTION AND CRYOGLOBULINEMIA

Chronic infection with hepatitis C virus (HCV) has now been firmly associated with the development of mixed cryoglobulinemia, and >90% of patients with cryoglobulin-associated renal disease are infected with HCV in some series (136). HCV infection has also been linked with the development of glomerular disease in the absence of cryoglobulins.

Presentation

Most patients with cryoglobulin-associated renal disease have proteinuria, hematuria, and an elevated serum creatinine. The presentation can be more fulminant, and some patients present with the full nephritic syndrome and with acute elevations in the serum creatinine. By the time patients present with renal disease, they usually have systemic manifestations of vasculitis. Common extrarenal signs and symptoms include palpable purpura, arthralgias, and weakness. Hypertension is also common. In patients with active disease the serum C4 level is almost always low (149). The cryocrit is quite variable but can usually be detected, and rheumatoid factor is usually present.

HCV infection, even in patients who do not have detectable cryoglobulins, has been linked to the development of MPGN (149,150). HCV has also been linked to MN, although this correlation has not been firmly established. One report also found that many HCV-infected patients with renal involvement may go undetected (151). In this study, 30 patients undergoing liver transplantation for HCV-induced cirrhosis underwent renal biopsy at the time of liver transplantation. None of the patients had detectable cryoglobulins, yet all but one had some type of glomerular pathology. Of 25 patients with glomerular IC deposits, 10 had a normal urinalysis, further suggesting that standard screening of patients with HCV infection may underestimate the degree of renal involvement.

Pathophysiology

Cryoglobulins are present in the majority of patients with HCV-associated glomerular disease (150). Most patients have type II cryoglobulinemia, in which the cryoglobulins contain a monoclonal antibody (usually an IgM) that binds to polyclonal IgG. Studies of biopsy tissue indicate that HCV complexes are present within the glomerular capillary walls (152). Furthermore, the IgM rheumatoid factor present in the cryoglobulins of HCV-infected patients can cause GN in mice when passively transferred (153). These findings suggest that cryoglobulins containing HCV cause IC-mediated injury of the glomerulus, perhaps because of the affinity of the IgM component for a glomerular target.

Prognosis and Treatment

Cryoglobulin-associated renal disease has a variable course. Approximately 10% to 15% of patients will enter spontaneous remission, and another 30% will have an indolent course with only a mild degree of renal insufficiency. Some patients, however, have acute nephritic flares (154). Treatment of the underlying HCV infection with IFN α and ribavirin can improve symptoms of cryoglobulinemia (155), and antiviral treatment can reduce proteinuria in patients who achieve a virologic response (156). The choice of antiviral treatment depends upon the hepatitis C genotype and a patient's renal function (157). Sofosbuvir is a recently approved drug that can achieve sustained virologic response with all of the different genotypes (158). Although patients with a GFR <30 mL/minute were not initially studied, subsequent reports have effectively used sofosbuvir in patients with advanced renal failure (159). Acute nephritic flares are not controlled by antiviral therapy. In this setting, aggressive therapy with plasmapheresis, corticosteroids, and cyclophosphamide may be effective at controlling the disease flare (160). Rituximab has also been reported to improve the serologic parameters and the degree of proteinuria (161).

HEPATITIS B VIRUS INFECTION

Chronic infection with hepatitis B virus (HBV) is most common in Asia and Africa, where the prevalence of HBV infection is the highest and where there is the highest incidence of vertical transmission. Acute infection with HBV can cause a serum sickness–like syndrome or

polyarteritis nodosa (PAN). The latter condition affects small or medium-sized arteries. The glomeruli may show ischemic changes in patients with PAN, and membranous and proliferative glomerular lesions can also occur. Chronic carriers of HBV can develop mesangial, subendothelial, and subepithelial IC deposits, and HBV-associated antigens are frequently detectable within the glomeruli (162). The mesangial and subendothelial deposits cause a MPGN pattern of injury. Subepithelial deposits cause a membranous pattern of disease and nephrotic syndrome, although concurrent mesangial and subendothelial deposits are also frequently present in these patients. HBV infection is also associated with the development of IgAN. Treatment of the HBV infection with IFN probably ameliorates the renal disease in some patients (163), and treatment with lamivudine has improved the renal disease in some case reports. Treatment with immunosuppressive medications can be considered in severe cases, such as in patients with RPGN or severe manifestations of PAN.

CHRONIC PARASITIC INFECTIONS

Glomerular disease may be seen in patients infected with several types of parasites. Acute IC–mediated GN may be seen in patients with malaria, including a chronic proliferative GN in patients infected with *Plasmodium malariae* (164). *Schistosoma mansoni* infections may cause IC-mediated GN and amyloidosis. Several other parasitic infections have also been associated with IC-mediated GN, presenting with proliferative or membranous patterns by light microscopy.

LUPUS NEPHRITIS

SLE is an autoimmune disease that can affect multiple different organs, including the skin, joints, lungs, and kidneys. Up to 60% of adults and 80% of children who are diagnosed with lupus will develop renal abnormalities at some point (165), but there is patient-to-patient variation in the nature of the renal disease. Aggressive immunosuppressive therapy has greatly improved the outcome in patients with lupus nephritis over the past several decades, and the impact of newer therapies on the overall prognosis is not yet known. Nevertheless, up to 30% of patients with renal involvement may go on to ESRD with long-term follow-up (166,167), and overall patient survival is worse in those with renal involvement (168). One of the great challenges in treating patients with lupus, therefore, is to identify those patients who will most benefit from aggressive

immunosuppression while also minimizing the toxicities of therapy.

Pathogenesis

The pathogenesis of lupus itself remains uncertain, but it likely involves genetic defects that cause a loss of tolerance to self-antigens. Patients with lupus frequently develop high-affinity autoantibodies to nuclear antigens, cytoplasmic antigens, platelets, and erythrocytes. Antibodies to dsDNA, other nuclear components, and α-actinin are associated with renal disease, and there is evidence that these antibodies are pathogenic (169). Antibodies to other glomerular proteins have also been identified, including antibodies to α-enolase, annexin A1, and annexin A2 (170,171). Antibodies to C1q are also commonly seen in the kidneys of patients with lupus nephritis, and these antibodies may exacerbate renal injury (172).

In patients with lupus nephritis, ICs may be found in mesangial, subendothelial, and/or subepithelial locations. ICs cause tissue inflammation by activating complement and through their interaction with Fc receptors on immune cells. The location of the ICs correlates with the light microscopic changes and with the clinical presentation. Mesangial ICs may cause mesangial expansion and hypercellularity, and patients typically have microscopic hematuria and subnephrotic range proteinuria. Subendothelial ICs cause an exudative lesion in the glomerulus. There is leukocyte infiltration and endocapillary proliferation. Subepithelial ICs tend to cause proteinuria and a membranous pattern of glomerular injury. IC deposition and inflammatory cells can also damage the tubules and blood vessels. Some patients develop renal injury caused by antiphospholipid antibody–mediated glomerular thrombosis.

Secondary Factors

Some drugs are associated with the development of a lupus-like syndrome. These patients develop ANA and antihistone antibodies, but anti-dsDNA antibodies and renal involvement are rare. Commonly implicated drugs include procainamide and hydralazine.

Presentation

Depending on the severity of the renal disease, patients may present with proteinuria (subnephrotic or nephrotic), hematuria (microscopic or gross), red blood cell casts, hypertension, edema, and an elevation in serum

creatinine. Patients can present with a pulmonary-renal syndrome, and some patients develop an RPGN. Although the clinical presentation may correlate with the histologic pattern (e.g., a nephritic pattern of disease with subendothelial deposits and endocapillary proliferation), the clinical findings do not accurately predict the histology or the prognosis.

Serologic Findings

Virtually all patients with lupus nephritis have a positive ANA. As mentioned above, anti-dsDNA antibodies may be pathogenic in lupus nephritis and are very specific. C3 and C4 levels are frequently depressed in patients with active disease. In some patients, serologic changes may predict disease flares (173). Persistently high anti-dsDNA antibodies or low C3 levels are also associated with a greater risk of disease flare and disease progression (174,175), but these tests do not reliably predict disease flares. Thus, although perturbations in the levels of these factors may prompt closer monitoring or even a repeat renal biopsy, there is no evidence to support altering treatment in an effort to normalize their levels.

Histologic Patterns

Because the clinical course of lupus nephritis is so variable and the medications used to treat the disease have many potential side effects, great effort has been made to identify patients who benefit from aggressive immunosuppression. The WHO classification scheme was first proposed in 1982 (176), and has been modified several times since then (177). This scheme includes six classes of glomerular involvement, with several different subclasses (Table 15-9). Several series have established the importance of the WHO classification for predicting patients' long-term outcomes (178). Equally important, this classification scheme has been used as a criterion for the selection of patients in most large clinical trials. To determine whether clinical trials apply to a particular patient, therefore, the patient's histologic class must be established. Other factors on the biopsy that may help identify which patients are at a high risk of progression include activity and chronicity indices (179). Over time, the histologic pattern of disease can change (167). For this reason, repeat biopsies are often necessary for optimal assessment of disease activity (180).

Prognosis

The course of lupus nephritis has improved in recent decades, but lupus is still a significant cause of CKD and ESRD (166,181). Important prognostic factors include the WHO histologic classification and disease activity and chronicity. In some reports, patients with WHO class IV disease were more likely to respond to treatment than those with WHO class III disease (182), perhaps due to different mechanisms of glomerular injury (183). In patients with severe proliferative disease, those who achieve and maintain remission with therapy have better long-term outcomes (182). A lower serum creatinine and lower urine protein excretion predict a response to therapy, and those patients who enter remission tend to show marked improvement within 4 weeks of initiating therapy. Urine protein excretion at 1 year is predictive of the long term prognosis (184). Several series have reported that black patients are less likely than white patients to respond to therapy (182,185). Patients who have flares of their renal disease, particularly nephritic flares, have a worse long-term outcome (186,187).

Table 15–9 Major Histologic Classes of Lupus Nephritis

Class	Description
Class I	Normal light microscopy, mesangial immune deposits.
Class II	Mesangial hypercellularity or matrix expansion, mesangial immune deposits.
Class III	Focal lupus nephritis. Active or inactive focal, segmental or global endocapillary or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations.
Class IV	Diffuse lupus nephritis. Active or inactive diffuse, segmental or global endocapillary or extracapillary glomerulonephritis involving >50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations.
Class V	Membranous lupus nephritis. Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial

	alterations. Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed.
Class VI	Advanced sclerosis (>90% of glomeruli globally sclerosed)

Modified from Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol*. 2004;15:241–250.

Treatment

Because of the variable course of lupus nephritis, the decision to treat an individual patient is based upon the overall risk of progression. In general, immunosuppressive treatment is instituted in patients with proliferative GN (classes III and IV) and in some patients with membranous GN (class V). Patients with mesangial disease are not usually treated with immunosuppressive drugs because of the benign long-term prognosis, and patients with advanced sclerosis are not treated because of the low likelihood of responding to therapy.

Several large randomized trials have demonstrated the efficacy of cyclophosphamide combined with corticosteroids for the treatment of proliferative lupus nephritis (188,189). Aggressive induction therapy with combined cyclophosphamide and corticosteroids effectively controls renal inflammation and induces remission. On the basis of these early studies, for many years the standard treatment of patients with WHO class III or IV lupus nephritis included induction with 6 monthly intravenous pulses of cyclophosphamide (0.5–1.0 g/m^2) and glucocorticoids (e.g., prednisone started at 1 mg/kg/d). Shorter induction protocols have also been effectively used in patients with mild disease (190).

More recently, several randomized controlled trials have now demonstrated that MMF is as effective, or possibly even superior to cyclophosphamide for treatment of proliferative lupus nephritis. Chan et al. first demonstrated that induction therapy with 1 g of MMF twice daily was as effective as oral cyclophosphamide for patients from Hong Kong (191). A multicenter randomized controlled trial in the United States also demonstrated that MMF was as effective as cyclophosphamide for treatment of patients with proliferative disease (the dose in this study was escalated to 1.5 g twice daily) (192).

After induction therapy, patients with proliferative GN should be switched to maintenance therapy protocols. Early studies demonstrated

that patients maintained with pulses of cyclophosphamide every 3 months had fewer disease flares and better long-term renal function than those who did not receive maintenance therapy (188). Regimens containing azathioprine, mycophenolate, and cyclosporine have also been tried as maintenance therapy. In a randomized trial comparing MMF with cyclophosphamide for maintenance therapy, MMF was more efficacious than cyclophosphamide (193). On the basis of these results, many patients are now maintained on MMF for 18 to 24 months after completing their induction protocol. MMF may be administered in doses of 1 to 2 g/day and tapered to 0.5 to 1 g/day in the third year of treatment. Low-dose prednisone is typically continued during the maintenance period (193).

Several alternative therapies may be of benefit in patients who do not respond to induction therapy or who have relapses. Several small nonrandomized studies have demonstrated that rituximab can induce complete or partial remissions in patients who have relapsed or failed to respond to conventional therapy, but the addition of rituximab to mycophenolate in a randomized controlled trial did not show added benefit (194). Cyclosporine has also been reported to induce remission in patients who either fail induction therapy or who relapse (195). The combination of mycophenolate with a calcineurin inhibitor may also be effective in patients with severe disease or with class IV+V disease (196,197). Therapy in resistant or relapsing patients can be changed (e.g., treatment with cyclophosphamide and corticosteroids can be changed to mycophenolate and corticosteroid) or rituximab can be added to their regimen.

Approximately 10% to 15% of patients with lupus nephritis have a pure membranous lesion (ISN/RPS class V), and the optimal therapy for this subgroup is not clearly defined. Most treatment studies for these patients have either been uncontrolled, or the studies have combined patients with proliferative and membranous patterns of disease. The newer classification system more clearly defines patients whose biopsies display both proliferative and membranous patterns, and most authors agree that the treatment of patients with combined lesions should be based on the proliferative component of their disease. For patients with a pure membranous lesion, a prospective study compared three different immunosuppressive regimens (198). This study demonstrated that intravenous cyclophosphamide and oral cyclosporine were effective at inducing remission in 60% and 83% of the patients, respectively. Both therapies were superior to alternate day prednisone, but there was a high rate of relapse in the cyclosporine group after therapy was stopped. Retrospective studies indicate that corticosteroids in combination with

cyclosporine, azathioprine, cyclophosphamide, or mycophenolate may be effective. The long-term benefit of these therapies on patient outcomes is still unclear.

ANCA-ASSOCIATED VASCULITIS

Vasculitis may involve vessels of any size. Small vessel vasculitis commonly involves the arterioles of the kidney and the glomerular capillaries, but these diseases can affect any vessel in the body. Small vessel vasculitis can be caused by IC deposition in the vessel wall in patients with diseases such as lupus and HSP. Vasculitis without IC deposition on tissue biopsy (termed "pauci-immune") is usually associated with circulating ANCA. The ANCA-associated vasculitides (AAV) include granulomatosis with polyangiitis (GPA, previously referred to as Wegener granulomatosis), microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis (EGPA, or Churg–Strauss syndrome), and renal limited vasculitis. AAV involving the kidney often causes rapid deterioration of renal function, and the glomerular lesion is commonly necrotizing and crescentic (199). The terms "crescentic GN" or "RPGN" are sometimes used interchangeably with the ANCA-associated diseases. However, IC-mediated vasculitides can also cause these clinical and morphologic presentations.

Pathogenesis

Serum ANCA may be identified by indirect immunofluorescence with ethanol-fixed neutrophils. Antibodies in the serum from ANCA-positive patients will react with the neutrophils in either a cytoplasmic (C-ANCA) or perinuclear (P-ANCA) pattern. The C-ANCA pattern is usually caused by antibodies directed against proteinase-3 (PR-3), and the P-ANCA pattern is usually caused by antibodies specific to myeloperoxidase (MPO) (200). PR-3 and MPO are proteins contained in the granules of neutrophils and the lysosomes of monocytes. Autoantibodies to lysosomal membrane protein-2 (LAMP-2), a protein present on neutrophils and endothelial cells, have also been identified in patients with AAV (201).

Although the diseases are referred to as pauci-immune, there is strong evidence that the ANCAs are pathogenic in AAV. In vitro studies have demonstrated that ANCAs bind to primed neutrophils and trigger activation of the cells (202), and passive transfer of anti-MPO antibodies in mice induces a crescentic, pauci-immune GN (203). The passive

transfer of anti-MPO antibodies from mother to newborn has also been reported to cause a pulmonary-renal syndrome in the child (204). ANCAs probably contribute to the development of vasculitis through direct effects on circulating neutrophils or by causing endothelial damage. Genetic and environmental (drugs, infections, chemicals) factors may also contribute to the development of AAV.

Presentation

All patients suspected of having AAV should undergo testing for ANCA with both indirect immunofluorescence and enzyme-linked immunosorbent assays (ELISAs) specific for PR-3 and MPO. The diseases that cause AAV are defined on the basis of the organs involved, their ANCA associations, and the presence or absence of granulomas on tissue biopsy (Table 15-10). These criteria are not absolute, so there is some overlap in the disease definitions. Other inflammatory and infectious diseases, such as bacterial endocarditis, are associated with the development of ANCA and should be considered in the differential diagnosis. Patients with AAV involving the kidney typically present with hematuria, subnephrotic range proteinuria, and a rapid rise in serum creatinine. Patients frequently have a nephritic presentation with diffuse necrosis and crescents on biopsy. C3 and C4 levels are usually normal or elevated. The other organ systems that are commonly involved upon presentation include the lungs and upper airways, ears, nose, throat, skin, neurologic system, and gastrointestinal tract. In addition to the involvement of these specific organs, patients with AAV often also have systemic symptoms such as weight loss and fevers. Patients with EGPA invariably have a history of asthma, and often present with eosinophilia.

Prognosis

Untreated, the 2-year survival for patients with AAV may be below 20%, and the prognosis for these diseases has been significantly improved by aggressive immunosuppressive protocols (179). With treatment, the long-term survival is now approximately 50% to 80%. Approximately 75% of those treated achieved at least partial remission (205), but approximately 30% of patients experience a relapse within several years of entering remission (206,207).

Treatment

Because of the poor prognosis of untreated disease, virtually all patients with AAV are treated with induction and maintenance protocols of immunosuppressive drugs, and the treatment protocols are similar for all of the diseases discussed above. Corticosteroids in combination with intravenous or oral cyclophosphamide has long been the standard treatment, although several recent randomized trials found that rituximab is as effective as cyclophosphamide for achieving remission (207–209). Patients may be treated with three daily pulses of methylprednisolone and are then usually treated with oral prednisone during the induction period. Plasma exchange may also benefit patients with severe disease, particularly those with significant renal dysfunction or are on dialysis at the time of diagnosis (210,211). Steroids are usually tapered off after patients achieve remission, and cyclophosphamide is continued for another 6 to 12 months. Azathioprine can be used to maintain patients in remission (212). Rituximab is also effective as a maintenance drug, and may be superior to azathioprine (206). MMF, on the other hand, appears to be less effective than azathioprine for maintaining remission (213).

Table 15–10 Clinical and Morphologic Features of the ANCA Associated Vasculitides

Disease	ANCA			Other Organs Commonly Involved	Biopsy Findings
	PR3-ANCA	MPO-ANCA	ANCA Negative		
Microscopic polyangiitis	40%	50%	10%	Skin, lungs, musculoskeletal, GI	Necrotizing glomerulonephritis and vasculitis. Pulmonary capillaritis.
Granulomatosis with polyangiitis (GPA, formerly Wegener disease)	75%	20%	5%	Skin, lungs, musculoskeletal, neurologic, GI	Necrotizing glomerulonephritis. Granulomas in respiratory tract and kidneys.
Eosinophilic granulomatosis with polyangiitis (EGPA, or Churg–Strauss syndrome)	10%	60%	30%	Skin, lungs, musculoskeletal, neurologic, GI	Necrotizing glomerulonephritis. Eosinophil rich and granulomatous inflammation in respiratory tract.
Renal limited vasculitis	20%	70%	10%	—	Necrotizing glomerulonephritis.

ANCA, antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; GI, gastrointestinal; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis.
From Nachman PH, Jennette JC, Falk RJ. Vasculitic diseases of the kidney. In: Schrier RW, ed. *Diseases of the Kidney and Urinary Tract*. 8th ed. Philadelphia: Saunders; 2007, with permission.

ANTI-GLOMERULAR BASEMENT MEMBRANE DISEASE

Anti-GBM disease, or Goodpasture disease, is an autoimmune disease caused by the development of autoantibodies directed against the GBM and the alveolar basement membrane. Goodpasture disease commonly presents as an RPGN and may be accompanied by pulmonary hemorrhage (pulmonary-renal syndrome).

Pathogenesis

Goodpasture disease is caused by antibodies (usually IgG, but occasionally IgA or IgM) with high affinity for two specific epitopes in the noncollagenous (NC1) domain of type IV collagen found in the basement membrane. These epitopes are usually sequestered within the collagen structure. Goodpasture disease is associated with environmental factors such as hydrocarbons and tobacco smoke, and it is believed that these insults may damage the GBM and expose the epitopes. Certain MHC alleles are associated with a greater risk of developing disease, and a loss of T-cell tolerance is probably also necessary for the development of the

antibody response. In animal models, disease can be caused by the passive transfer of either the antibodies or T cells, both of which can trigger an inflammatory response in the glomerulus. There is evidence of a genetic susceptibility in patients with HLA DR15 and DR4 (214).

Presentation

In 1919, Ernest W. Goodpasture reported a patient who presented with renal failure and pulmonary hemorrhage, and the name Goodpasture syndrome was later coined for patients who present with RPGN and pulmonary hemorrhage (215). Diseases causing systemic vasculitis (such as lupus and AAV) can also cause Goodpasture syndrome, and anti-GBM antibodies cause only about 30% of the same. Furthermore, patients with Goodpasture disease (e.g., pulmonary or renal disease specifically caused by anti-GBM antibodies) may present with isolated pulmonary or renal failure. Nevertheless, Goodpasture disease usually manifests with the acute onset of hemoptysis and a nephritic syndrome. The disease is most common in men in their 20s and 30s, and a second peak incidence is seen in people in their 60s. Urinalysis reveals erythrocytes, red cell casts, and subnephrotic range proteinuria, and chest X-rays may reveal diffuse alveolar hemorrhage. Patients with Goodpasture syndrome also usually have anemia.

Anti-GBM antibodies in the serum of patients with Goodpasture disease can be detected by ELISA, although the assay can be negative in patients with low antibody titers or with isolated pulmonary disease (216). On biopsy, Goodpasture disease usually causes a proliferative GN with crescents and areas of necrosis by light microscopy, similar to the appearance of AAV. In most cases, immunofluorescence microscopy reveals the linear deposition of immunoglobulin along the GBM (Fig. 15-6).

Prognosis and Treatment

There is some reported variability in the rate at which Goodpasture disease progresses, but in general, it is a fulminant disease that must be identified and treated promptly. Indeed, patients with mild or atypical symptoms may fare worse because of delayed treatment. Before the development of effective treatment, the patient and renal survival was dismal (217). Current therapy of patients with Goodpasture disease involves plasma exchange to remove circulating anti-GBM antibodies, cyclophosphamide

to prevent new antibody formation, and corticosteroids to dampen tissue inflammation. Rituximab has also been used in some patients and has been reported to help patients refractory to conventional therapies (218). The titer of anti-GBM antibodies correlates with disease progression, and plasma exchange should be continued while the antibodies remain detectable. Infection is a major cause of mortality in patients with Goodpasture disease, so patients receiving cyclophosphamide should receive *Pneumocystis jirovecii* pneumonia prophylaxis (e.g., Bactrim), and neutrophil counts should be monitored.

Figure 15–6 Fluorescent antibody staining of the glomerulus, showing discrete linear deposition of immunoglobulin, which is characteristic of anti-GBM–mediated glomerulopathies and Goodpasture disease. The immunoglobulin deposits represent autoantibody that has reacted with a native glycoprotein (noncollagen) constituent of the GBM.

THROMBOTIC MICROANGIOPATHY

"Thrombotic microangiopathy (TMA)" is a descriptive term for a morphologic lesion characterized by platelet thrombi occluding the microvasculature of various organs. Various diverse disorders share this common pathology, including thrombocytopenic purpura (TTP), diarrhea-associated and atypical hemolytic uremic syndrome (d/aHUS), and

scleroderma renal crisis. TMAs are thought to be triggered by microvascular endothelial cell injury that then leads to platelet and fibrin thrombi.

On light microscopy, the glomeruli frequently have platelet and fibrin clots. These clots can extend into the arterioles and even occasionally into larger vessels that can show necrosis with intimal swelling, mucoid change, and intimal proliferation. Mesangiolysis can occur. Glomeruli may only show evidence of ischemia with corrugation of the GBM and retraction and collapse of the glomerular tuft. Segmental glomerular necrosis may be seen. Secondary changes late in the course of the disease include reduplication of the GBM often similar in appearance to an MPGN pattern of injury. C4d and IgM are sometimes seen by immunofluorescence microscopy (219). EM shows swollen endothelial cells that frequently appear to have detached from their basement membranes.

Pathogenesis

Idiopathic TTP is often secondary to a deficiency in ADAMTS13 (A Disintegrin-like and Metalloprotease with ThromboSpondin type 1 repeats). ADAMTS13 is synthesized primarily by the liver and endothelial cells. Its main function is to cleave ultra-large von Willebrand factor (vWF) multimers that are released from endothelial cells. vWF supports platelet adhesion and aggregation at sites of shear stress. ADAMTS13 deficiency allows circulating ultra-large vWF to persist causing platelet aggregation and clumping leading to TTP. Congenital ADAMTS13 deficiency has been described and is the result of an inactivating mutation. Acquired deficiency is caused by inhibitory antibodies, which are found in 50% to 94% of patients with undetectable ADAMTS13 (220). Noninhibitory antibodies that may play a role in increased clearance or endothelial cell binding have also been described (221).

Diarrhea-associated HUS is most commonly caused by shiga toxin–producing *Escherichia coli* O157:H7. The shiga toxin binds the glycolipid cell surface receptor Gb3, is endocytosed, and subsequently binds to the 60S subunit of ribosomes inhibiting protein synthesis and injuring cells. This endothelial cell injury exposes the underlying basement membrane causing activation of platelets and the coagulation cascade (222). In atypical HUS mutations in complement, regulatory proteins and activating proteins lead to uncontrolled complement activation and subsequent cell injury (223). The secondary causes of TTP–HUS likely lead to a final

common pathologic pathway via endothelial cell injury. Atypical HUS can also develop in young children with variants in the gene for diacylglycerol kinase epsilon (DGKE) (224).

Secondary Causes

TMAs have been associated with a number of different medications, including cyclosporine, tacrolimus, sirolimus, quinine, OKT3, mitomycin C, cisplatin, bleomycin, gemcitabine, cyclophosphamide, antivascular endothelial growth factor (VEGF) antibodies, valacyclovir, oral contraceptives, ticlodipine, and clopidogrel (225). TMA can develop after hematopoietic stem cell transplantation (226). TTP–HUS associated with pregnancy is also well described, occurring either in isolation or with severe preeclampsia/HELLP syndrome. A large percentage of patients with postpartum HUS have rare variants in genes of the complement system, suggesting that this is a complement-mediated process (227). TMAs can also occur with HIV, malignant hypertension, the antiphospholipid antibody syndrome, lupus, scleroderma, pneumococcal infection, and malignancies.

Presentation

The Oklahoma TTP–HUS registry reports the incidence of suspected TTP at 11 cases/million population per year. Incidence is greater for women and blacks (228). TMAs share a number of clinical and serologic features; all have microangiopathic hemolytic anemia—demonstrated by elevated lactate dehydrogenase levels (also from tissue ischemia), decreased haptoglobin, and elevated indirect bilirubin—thrombocytopenia, and secondary organ involvement. The classic pentad for TTP had been fever, microangiopathic hemolytic anemia, TTP, renal failure, and central nervous system involvement. This severe stage of the disease is rarely seen anymore, however, as treatment is usually initiated much earlier. HUS typically presents with more severe renal failure and milder thrombocytopenia than TTP, but significant overlap occurs between the two disorders. The distinction of TTP and HUS is usually based on a low level of ADAMTS13 activity in the plasma (<10%).

Diarrhea-associated HUS occurs most commonly in children and presents with bloody diarrhea. *E. coli* O157:H7 accounts for over 60% of cases. In 2011, a severe outbreak in Europe caused by a shiga toxin–producing strain of *E. coli* O104:H4 affected more than 3,000 patients

(229). Atypical HUS is associated with a number of different mutations in complement related proteins that can be detected by specific assays, including factor H, membrane cofactor protein (CD46), factor I, C3, and factor B. In atypical HUS the serum C3 level is often low.

Prognosis

Diarrhea associated HUS is usually a self-limited disease with a good prognosis. The 2011 outbreak was particularly severe, however, and renal replacement therapy was needed to support a large percentage of the patients (230). Idiopathic TTP that is treated promptly has a good prognosis, but relapse rates are high with almost 50% having at least one recurrence. Young age and low ADAMTS13 activity are risk factors for recurrence (231). Untreated, atypical HUS has a poor prognosis with many patients reaching ESRD. Recurrence after transplantation is very common, except for patients with CD46 mutations as the donor kidney will correct the genetic defect unless it comes from a family member with the same mutation (232).

Treatment

Suspected TTP should be treated with plasma exchange performed daily until the platelet count has normalized and hemolysis ceased (233). Corticosteroids and rituximab may also be beneficial in TTP (234,235). Eculizumab (a monoclonal antibody that prevents the cleavage of C5 during complement activation) has been approved for the treatment of complement-mediated atypical HUS (236). Eculizumab appears to be superior to plasma exchange for this disease, and is the treatment of choice. For shiga toxin–mediated HUS, it is not clear whether plasma exchange and eculizumab are beneficial. Treatment, therefore, is primarily supportive. For secondary causes of TMA, the triggering event should be removed, if possible. The exception to this is pregnancy-induced HUS. Given that a large percentage of these patients harbor mutations in complement-associated genes, treatment with eculizumab should be considered.

GLOMERULAR INVOLVEMENT IN OTHER MULTISYSTEM DISEASES

Other autoimmune diseases have been associated with the development of

glomerular disease. This is not unexpected as one might predict that diseases in which there are high levels of circulating rheumatoid factors and ICs would cause IC deposition in the kidney. Patients with mixed connective tissue disease may develop anti-dsDNA antibodies, and GN may be common in these patients. Rheumatoid arthritis (RA) is associated with the development of secondary amyloidosis, particularly in those who have had poorly controlled disease for prolonged periods (237). Cases of MN, mesangial IC deposition, and even proliferative disease have been reported in patients with RA. In some reports, the patients may have had lupus, so the association and overall incidence is uncertain. The treatment of RA with gold or penicillamine, however, has been clearly linked with the development of MN. IC-mediated GN has been reported in patients with Sjögren syndrome disease, ankylosing spondylitis, Beçhet disease, and polymyositis.

Monoclonal Immunoglobulin–Related Diseases

Plasma cell dyscrasias result from a clonal expansion of malignant plasma cells that secrete a monoclonal immunoglobulin. In healthy adults, plasma cells also synthesize an excess of light chains that then get freely filtered by the glomerulus and catabolized by proximal tubular cells. The uptake of light chains is constant and occurs via binding to the megalin–cubilin complex followed by the endosomal/lysosomal degradation of the proteins, ultimately leading to the return of the free amino acids to the circulation (238–241). Despite this process, small amounts of polyclonal free light chains do make their way into the urine at a concentration of about 2.5 *m*g/mL. In multiple myeloma and other plasma cell dyscrasias, the amount of light chains produced and filtered exceeds the maximal reabsorptive capacity of the proximal tubular cells and make their way into the urine at much higher concentrations, where they are also referred to as Bence–Jones proteins.

The toxicity of light chain proteinuria depends upon the specific characteristics of the light chain. Certain toxic light chains are able to self-aggregate forming high molecular weight polymers that are then able to deposit in tissue or form tubular casts after binding to Tamm–Horsfall proteins (242), whereas some have toxicity based on the variable region of the light chain molecule.

Light chains are known to cause a number of different kidney diseases, some affecting the tubulointerstitium (see myeloma cast nephropathy,

proximal tubule dysfunction, interstitial nephritis in the appropriate chapters) and others the glomerular compartment.

AMYLOID

The amyloidoses are a collection of both acquired and hereditary protein folding disorders in which deposits of abnormally folded proteins form fibrils and lead to tissue destruction and disease progression. Inherent in all of the amyloid proteins is the *β*-pleated sheet secondary structure.

The most common type of amyloid in the western world is primary amyloidosis (AL) where the amyloid fibril is derived from an immunoglobulin light chain. Immunoglobulin heavy chain amyloid (AH) is much less common. AL and AH are typically associated with plasma cell dyscrasias. Secondary amyloidosis (AA) is more common in the developing world and occurs in patients with chronic inflammatory conditions, most commonly RA and other connective tissue diseases, familial Mediterranean fever, inflammatory bowel disease, and chronic infections. Its precursor protein, the apolipoprotein serum amyloid A (SAA), is an acute phase reactant. In the hereditary amyloidoses, an inherited gene mutation creates an amyloidogenic protein that triggers the disease. The kidney is one of the most frequent sites of both AL and AA amyloid fibril deposition with slightly less frequent involvement in hereditary amyloid (243).

The light microscopy findings of amyloid appear as amorphous acellular, pale eosinophilic material in the mesangium and capillary loops. Amyloid stains positive with Congo red with apple green birefringence under polarized light. Immunofluorescence usually shows positivity in the affected areas when staining for the responsible light chain; however, false negatives may occur in close to 30% of patients (244). Immunohistochemistry can be used for specific amyloidogenic proteins, such as AA. EM shows randomly arranged fibrils 10 to 12 nm in size (245).

Pathogenesis

The pathogenesis of AL amyloid involves the formation of insoluble fibrils from a monoclonal immunoglobulin light chain produced by a modestly infiltrated bone marrow plasma cell clone. The *l*-light chain isotype is prevalent in AL. It is postulated that certain germ line genes for *λ*-light chains have enhanced propensity to aggregate (246). When mutations

cause amino acid substitutions, the proteins may have less thermodynamic stability and have different hydrophobic and electrostatic interactions, giving them an even greater propensity to form fibrils (246–248). This abnormal *l*-light chain is believed to interact with a phenotypically changed mesangial cell via a receptor-light chain internalization process; the initiation of monoclonal immunoglobulin deposition disease is thought to occur in the same way. Studies indicate, however, that the initial trafficking of light chains isolated from patients with AL versus those with monoclonal immunoglobulin deposition disease is different, thus leading to divergent patterns of light chain deposition. The light chains in AL amyloid are transported to lysosomes leading to fibril formation. The fibril deposits slowly replace extracellular matrix through decreased synthesis of mesangial matrix via a lack of TGF-β and its increased degradation mediated by upregulated expression of matrix metalloproteinases without concomitant upregulation of tissue inhibitors of metalloproteinases (249–252).

AA amyloid develops after a lengthy inflammatory response with overproduction of SAA by pro-inflammatory cytokines. SAA is proteolytically processed to AA protein in macrophages, which then release the carboxy terminal end of the now amyloidogenic protein into the extracellular space where it is thought to interact with mesangial cells ultimately forming fibrils and disease.

Presentation

Renal AL/AH and AA amyloid typically present with varying degrees of proteinuria and renal insufficiency, depending on the extent of renal involvement. Hematuria is common. Other organ systems are typically involved.

Race and sex do not appear to be a factor, with most patients presenting in their sixth decade. The majority of patients with AL amyloid will have monoclonal light chains found in the urine and blood. Approximately 10% of those with AL amyloid will have multiple myeloma.

Prognosis

AL amyloidosis has a poor long-term prognosis without treatment, and the median survival is only 4 to 6 months. Factors that influence the renal response to therapy are the degree of baseline proteinuria, with low

baseline proteinuria levels predicting a more favorable renal response. AA amyloid has a better prognosis with the 10-year survival being close to 20%. Mortality is affected by the progression to ESRD, infections, and other organ system involvement.

Treatment

Elimination of the monoclonal protein and the plasma cell clone is the goal of treatment for AL amyloid. Those patients eligible for HCT are typically offered such treatment. Those patients not eligible for HCT are treated with various regimens of melphalan and steroids. The process of following response to therapy has been simplified with the advent of the serum-free light chain assay that has a significantly higher sensitivity than serum protein electrophoresis and immunofixation.

AA amyloid is treated with an emphasis at reducing the ongoing underlying inflammatory state. Colchicine has been used to treat AA amyloid associated with familial Mediterranean fever, and it has been demonstrated that treatment decreases systemic symptoms and stabilizes renal function. Colchicine has also been used anecdotally in AA amyloid associated with other chronic inflammatory conditions. Anti-cytokine biologic therapy is under investigation.

MONOCLONAL IMMUNOGLOBULIN DEPOSITION DISEASE

In patients with plasma cell dyscrasias, the glomerulus is in continual contact with abnormal free light chains. One consequence of light chain proteinuria is the development of the monoclonal immunoglobulin deposition diseases (MIDD). MIDD are classified as light-chain deposition disease (LCDD), light- and heavy-chain deposition disease, and heavy-chain deposition disease. Light microscopy demonstrates prominent nodular sclerosing glomerulopathy. Glomeruli can be enlarged with a diffuse and nodular expansion of the mesangial matrix. Basement membrane thickening can be seen, as can membranoproliferative features. Findings of myeloma cast nephropathy and amyloid in addition to MIDD have also been reported (253). Congo red stain is negative in MIDD. By immunofluorescence, light and/or heavy chain deposits can be found along the glomerular and tubular basement membranes, in the mesangium, renal vasculature, and interstitium. EM shows dense deposits within the glomerular and vascular basement membrane and external to tubular

basement membranes (254).

Pathogenesis

The initiation of LCDD begins with the interaction of structurally abnormal light chains, usually κ-I and κ-IV light chains, with the mesangial cell of the glomerulus. Amino acid substitutions within the variable region of light chains change the structure of the molecule often introducing hydrophobic residues and regions now able to be posttranslationally modified (255). These abnormal light chains interact with receptors present on mesangial cells causing them to transform into a myofibroblastic phenotype. Activation of growth factors, cytokines, and alteration in mesangial matrix expression leads to the generation of nodular glomerulosclerosis in a process similar to that described for AL amyloid (251).

The abnormal heavy chain molecules that cause disease are described as having a deletion of the CH1 domain of the heavy chain. Lack of this region prevents the complete construction of the immunoglobulin molecule leading to the secretion of free heavy chains from plasma cells (256). How these molecules lead to disease is not understood.

Presentation

MIDD typically presents with proteinuria, renal insufficiency, and hypertension. Nephrotic range proteinuria and microscopic hematuria are not uncommon. A monoclonal spike can be found in the serum and/or urine protein electrophoresis of most patients. Extrarenal deposition can occur in the liver, heart, and peripheral nervous system. Race and sex do not appear to be important factors. Most patients present in their sixth decade. The majority of patients presenting with MIDD have an underlying multiple myeloma/monoclonal gammopathy of undetermined significance or other lymphoproliferative disease; however, isolated MIDD has been reported (253,257). Of the MIDD, LCDD is by far the most prevalent.

Prognosis

The majority of patients diagnosed with MIDD reach ESRD within 2 to 4 years (253,257). Age and serum creatinine at presentation are the major predictive factors for the development of ESRD (257). MIDD does recur

in transplanted kidneys if the primary disorder is not effectively treated.

Treatment

There is no definitive treatment for MIDD. Most treatments are similar to those used to treat multiple myeloma, including regimens of melphalan/prednisone, vincristine/adriamycin/dexamethasone, steroids, and vincristine/cyclophosphamide/melphalan/prednisone (253).

CRYOGLOBULINEMIC GLOMERULONEPHRITIS

Cryglobulins are immunoglobulins that precipitate reversibly when cooled to ≤37°C. Type 1 cryoglobulins are composed of a monoclonal population of immunoglobulins, mainly IgG and are strongly associated with lymphoproliferative diseases such as leukemia, lymphoma, and plasma cell dyscrasias. Type II cryoglobulins are a mixture of polyclonal immunoglobulins in association with a monoclonal immunoglobulin with rheumatoid factor activity, and are usually associated with viral infections. Type III cryoglobulins are also polyclonal immunoglobulins without a monoclonal component and are seen in connective tissue diseases.

Cryoglobulinemic GN is generally an IC-mediated disease and is usually associated with the membranoproliferative pattern of injury by light microscopy; however, type 1 cryoglobulinemia may produce a less inflammatory renal reaction characterized by thrombosis and hypocellular lesions by light microscopy. Patients typically present with proteinuria, hematuria, and renal insufficiency.

WALDENSTRÖM MACROGLOBULINEMIA

Waldenström macroglobulinemia may have renal involvement. When present, it is associated with glomerular lesions consisting of large aggregates of the IgM paraprotein in glomerular capillary loops resembling thrombi. Acute renal failure may ensue. Amyloidosis may also complicate the picture. Therapy with chlorambucil and prednisone is beneficial.

FIBRILLARY AND IMMUNOTACTOID GLOMERULONEPHRITIS

Fibrillary and immunotactoid glomerulonephridities are uncommon causes

of GN occurring in <1% and <0.1% of native renal biopsies, respectively (258). Fibrillary glomerulonephritis (FGN) was first described in 1977 and initially labeled “amyloid like” because of the EM findings of organized electron-dense randomly deposited fibrils that failed to stain with Congo red (259). Since then, it has been characterized pathologically primarily by EM by the deposition in glomeruli of randomly arranged, nonbranching fibrillar immunoglobulin deposits that generally range in size between 18 and 22 nm. These fibrils stain via immunofluorescence for immunoglobulin, light chains, and C3. The light microscopy findings of FGN are heterogeneous and can show proliferative, membranoproliferative, mesangial proliferative, and even crescentic patterns of injury (260,261). Essential to the diagnosis is the absence of reactivity with Congo red.

Immunotactoid glomerulonephritis (ITG) is similar in many ways to FGN. It is Congo red negative with nondiagnostic light microscopy findings. By EM, however, ITG has microtubular immunoglobulin deposits >30 nm in size, which are often hollow and arranged in parallel arrays (260,261). Experts argue whether these two morphologically different entities should be considered a single disease process or if they have significant clinical and immunopathologic differences to merit differentiation.

Presentation

Both FGN and ITG typically present with nephrotic range proteinuria, hematuria, and renal insufficiency. More than 90% of patients are white. Some studies indicate that there is a slight female preponderance. The mean age at the time of diagnosis is around 50 years (260,262). Patients with ITG are more likely than those with FGN to have an underlying leukemia, lymphoma, or dysproteinemia. Both disorders have been associated with hepatitis C. Proponents of the theory that FGN and ITG are indeed a different morphologic expression of the same disorder argue that patients with underlying dysproteinemias should be excluded from a diagnosis of ITG.

Prognosis

Almost half of all patients with FGN or ITG develop ESRD within 2 to 6 years. The rate of disease progression has been linked to light microscopy findings with patients having severe proliferative disease progressing the

fastest and those with a membranous pattern of disease progressing slower (258). Both FGN and ITG have been reported to recur in transplanted kidneys.

Treatment

There is no specific treatment for FGN or ITG. Patients should receive nonspecific treatments such as control of blood pressure and proteinuria with renin–angiotensin system inhibitors. There are reports of the use of cytotoxic agents, prednisone, plasmapheresis, and NSAIDs with variable results (261). Some experts advocate directing treatment on the basis of light microscopy findings (258). Rituximab has been reported to be effective in a small number of patients (263). If an underlying lymphoproliferative disorder is found, treatment should be aimed at the primary disorder.

Glomerulopathies Associated with Metabolic, Biochemical, or Heredofamilial Disease

DIABETIC NEPHROPATHY

DN is the most common cause of CKD in the United States. Type 1 diabetes is caused by disorders of pancreatic β-cell destruction, whereas type 2 diabetes is caused by insulin resistance. Type 1 diabetes accounts for approximately 10% of patients with diabetes, whereas type 2 accounts for 90% of those with the disease.

The interpretation of epidemiologic studies in diabetes is challenging as many earlier studies were performed in an era without the aggressive diabetic management used today. That being said, approximately 25% of type 1 diabetic patients will have microalbuminuria (persistent albumin excretion between 30 and 300 mg/24 hours) after a mean duration of diabetes of 15 years and approximately 15% will progress to overt nephropathy manifested by proteinuria >300 mg/24 hours (264,265). The risk of DN in type 2 diabetic patients is almost equivalent to that of type 1 diabetic patients (266,267). Diabetic patients who have no proteinuria after 20 years of disease have a low risk of developing renal disease. Studies have also demonstrated that DN presenting with impaired renal function can occur in the absence of overt albuminuria, including those with normal levels of urinary protein (268,269).

In the setting of aggressive blood glucose control and blood pressure management, renal prognosis seems to have improved with <10% of patients with overt proteinuria progressing to ESRD (270,271).

A number of different risk factors have been determined which appear to influence the development of DN. These include poor glycemic control, poor blood pressure control, obesity, and smoking. African Americans and Mexican Americans also develop DN more frequently; however, it is difficult to eliminate confounding factors such as socioeconomic status from these studies. Age at the time of diagnosis may also be important. The degree of glomerular hyperfiltration increases the risk of developing DN (272). There also appears to be a genetic component to the development of DN with studies suggesting a role for the ACE genotype, the angiotensin-II type 2 receptor gene, and the aldose reductase gene.

Retinopathy is almost always present in type 1 diabetic patients with DN (273). Type 2 diabetic patients have a less predictable relationship with approximately 50% of patients with DN having retinopathy (273,274).

DN affects all of the compartments of the kidney including the glomeruli, vessels, interstitium, and tubules. The first change in the course of DN is hypertrophy of the glomeruli followed by thickening of the glomerular basement and tubular basement membranes and an increase in the mesangial matrix (275). The nodular lesions of DN, also called Kimmelstiel–Wilson nodules, begin in the center of the mesangial region of a segment (Fig. 15-7). Arteriosclerosis is often present as well. Arteriolar hyalinosis at the glomerular hilum typically affects both the afferent and the efferent vessel. Atrophic tubules and interstitial fibrosis develop as disease worsens. EM shows thickened basement membranes and mesangial expansion.

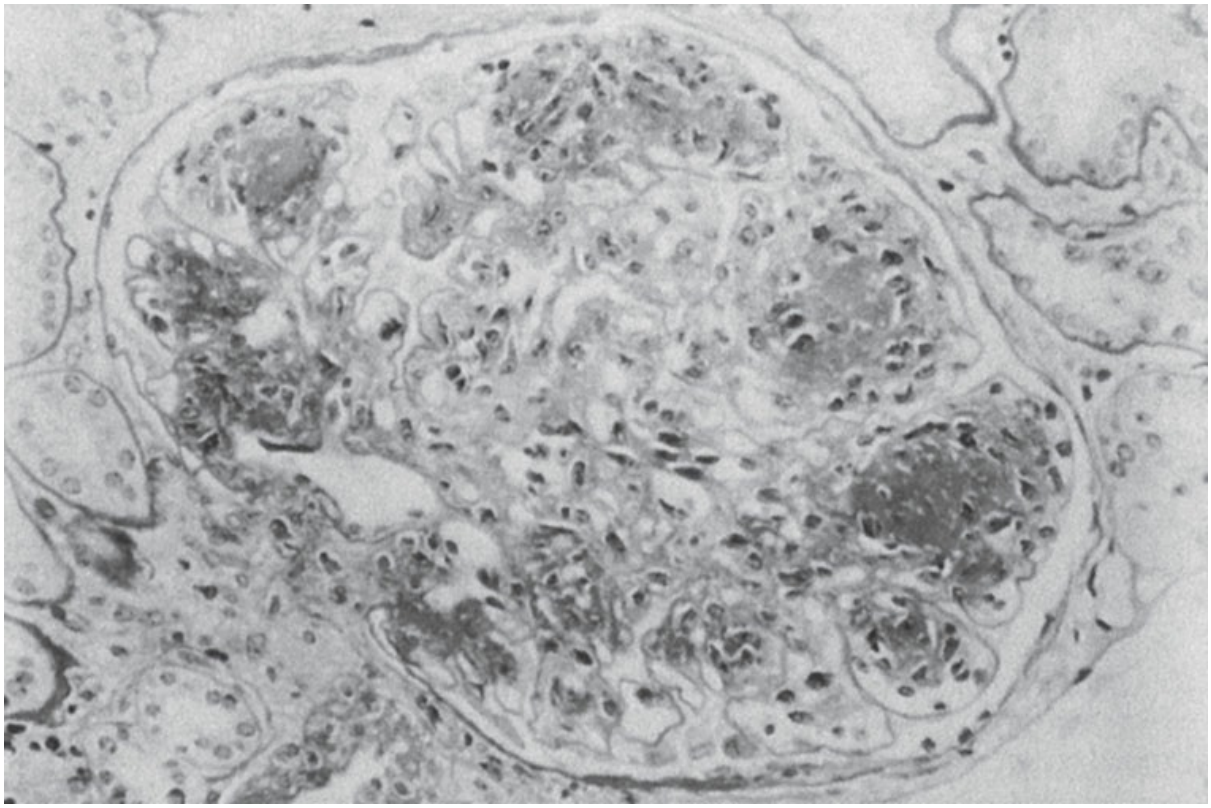

Figure 15–7 Light microscopic appearance of nodular diabetic glomerulosclerosis. Note the relatively acellular intercapillary nodule and diffuse increase in mesangial matrix. (Courtesy of Dr. Arthur H. Cohen.)

The Renal Pathology Society has developed a classification of type 1 and type 2 DN. Class I findings are isolated glomerular basement membrane thickening, class II findings are mesangial expansion, class III included at least one Kimmelstiel–Wilson lesion, and class IV demonstrates >50% global glomerulosclerosis. The severity of the interstitial fibrosis and vascular changes is also to be commented on. Like many classification systems, the clinical utility of this scheme is unknown.

Pathogenesis

The pathogenesis of DN is a complicated series of events initiated by metabolic factors and then perpetuated by mediator systems. GFR is increased early in the course of diabetes, in part due to increases in glomerular plasma flow, oncotic pressure, transcapillary pressure, and the glomerular ultrafiltration coefficient (276). Other studies have also implicated elevated levels of growth hormone and insulin-like growth factor as having a role in triggering hyperfiltration. Evidence that glomerular hypertension and hyperfiltration are important in DN has also been provided by many studies showing the benefits of blockade of the

renin–angiotensin system. Antagonizing the profibrotic effects of angiotensin II may also be a significant factor in benefits observed with these agents (277).

Hyperglycemia stimulates mesangial cell matrix production (278,279) and mesangial cell apoptosis (280). Hyperglycemia also causes glycosylation of proteins and generation of advanced glycosylation end products that further exacerbate disease through collagen cross-linking (281). Hyperglycemia also increases the expression of TGF-β, VEGF, and other cytokines in the glomeruli and matrix proteins that stimulate mesangial matrix accumulation (282,283).

Presentation

DN presents initially with microalbuminuria defined as protein excretion between 30 and 300 mg/24 hours. Renal function is preserved early on in the course of the disease. Hypertension is common. Microscopic hematuria may be present. Patients who present later in the course of the disease may have the nephrotic syndrome, and renal function may be significantly impaired.

Prognosis

The natural history of DN is a steady decline in GFR ranging from 1 to 24 mL/min/y associated with an increase in proteinuria and blood pressure (284). With aggressive blood pressure and glucose control, this rate of decline can be significantly improved. There is evidence that microalbuminuria can revert back to normal urinary protein excretion with good glucose and blood pressure control as well.

Treatment

The optimal therapy of DN is being investigated aggressively. The goals are to adequately treat blood pressure to a level <130/80 mm Hg, minimize proteinuria with a target goal of 500 to 1,000 mg/day, and control blood glucose. Lipids should be treated to guideline levels.

Blood pressure control and proteinuria reduction should include RAAS inhibition. These medications have been shown to slow the rate of disease progression (285). The majority of patients will need additional antihypertensive medications to obtain adequate control in addition to restricted sodium intake; diltiazem or verapamil can produce a further

reduction in protein excretion, which may correlate with further protection against progression of renal disease, whereas a loop diuretic is typically required in patients with edema or renal insufficiency. Mineralocorticoid receptor antagonism may also be used to improve blood pressure control and proteinuria, but in some patients, the risk of hyperkalemia is high. Experts continue to debate whether ACE inhibitors and ARBs should be used concurrently in order to maximize RAAS blockade.

ALPORT SYNDROME

Alport syndrome (or hereditary nephritis) is an inherited progressive form of glomerular disease often associated with sensorineural hearing loss and ocular lesions. The prevalence of the disease is estimated at approximately 1 in 50,000 live births. Alport syndrome develops in the setting of defects in type IV collagen, the primary structural component of the GBM. Six different genes have been identified on three different chromosomes; the translated gene products—type IV collagen molecules—interact with each other in complex mechanisms forming a network within basement membranes (286). When an abnormal protein is present, it disrupts the orchestrated development of basement membranes and leads to the Alport syndrome phenotype.

The genetics of Alport syndrome are heterogeneous (286). Over 80% of cases are X-linked and arise from mutations in the *COL4A5* gene on the X chromosome. Autosomal recessive Alport syndrome accounts for 15% of cases and results from homozygous or compound heterozygous mutations in the *COL4A3* or *COL3A4* gene. Approximately 5% of Alport syndrome cases display autosomal dominant inheritance and arise from heterozygous mutations in the *COL4A3* or *COL4A4* gene. It is unclear why some patients with heterozygous mutations develop Alport syndrome, whereas others develop the usually benign thin basement membrane disease.

The renal manifestations of Alport syndrome include either microscopic or gross hematuria, proteinuria, hypertension, and progression toward ESRD in males with X-linked disease and males and females with either autosomal recessive or dominant disease. Women with X-linked Alport syndrome are carriers of the disease and most have hematuria. Rarely more progressive disease can occur. The diagnosis of Alport syndrome is made by skin or renal biopsy; however, often times a positive family history makes tissue diagnosis unnecessary. Molecular genetic testing is also available. In a renal biopsy specimen, the light microscopy

is usually normal in the early course of the disease; however, at later stages, glomerulosclerosis and interstitial fibrosis may be present. Immunofluorescence is usually nonspecific unless special studies for type IV collagen are done. EM shows irregular thinned and thickened areas of the GBM with splitting and an irregular, multilaminated appearance of the lamina densa (Fig. 15-8). A skin biopsy for the diagnosis of X-linked Alport syndrome is done using a monoclonal antibody against the collagen α-5 (IV) chain, the protein product of *COL4A5* (287). If the protein is absent in a male or is clearly mosaic in a female, a diagnosis of Alport syndrome can be made without further testing. If the protein is present, then a diagnosis of autosomal recessive Alport syndrome is possible or a mutation in *COL4A5* may be present that allows deposition of a functionally abnormal but antigenically normal α-5 (IV) chain. The possibility of another disorder must also be considered.

Treatment of Alport syndrome is supportive. In patients with hypertension or proteinuria, RAAS inhibitors are recommended. There are some reports on the use of cyclosporine; however, most experts do not use cyclosporine at this time (288). Alport syndrome does not recur in transplanted kidneys, but de novo anti-GBM antibody disease develops in approximately 3% of transplanted males with antibodies directed against the newly introduced type IV collagen molecule (289).

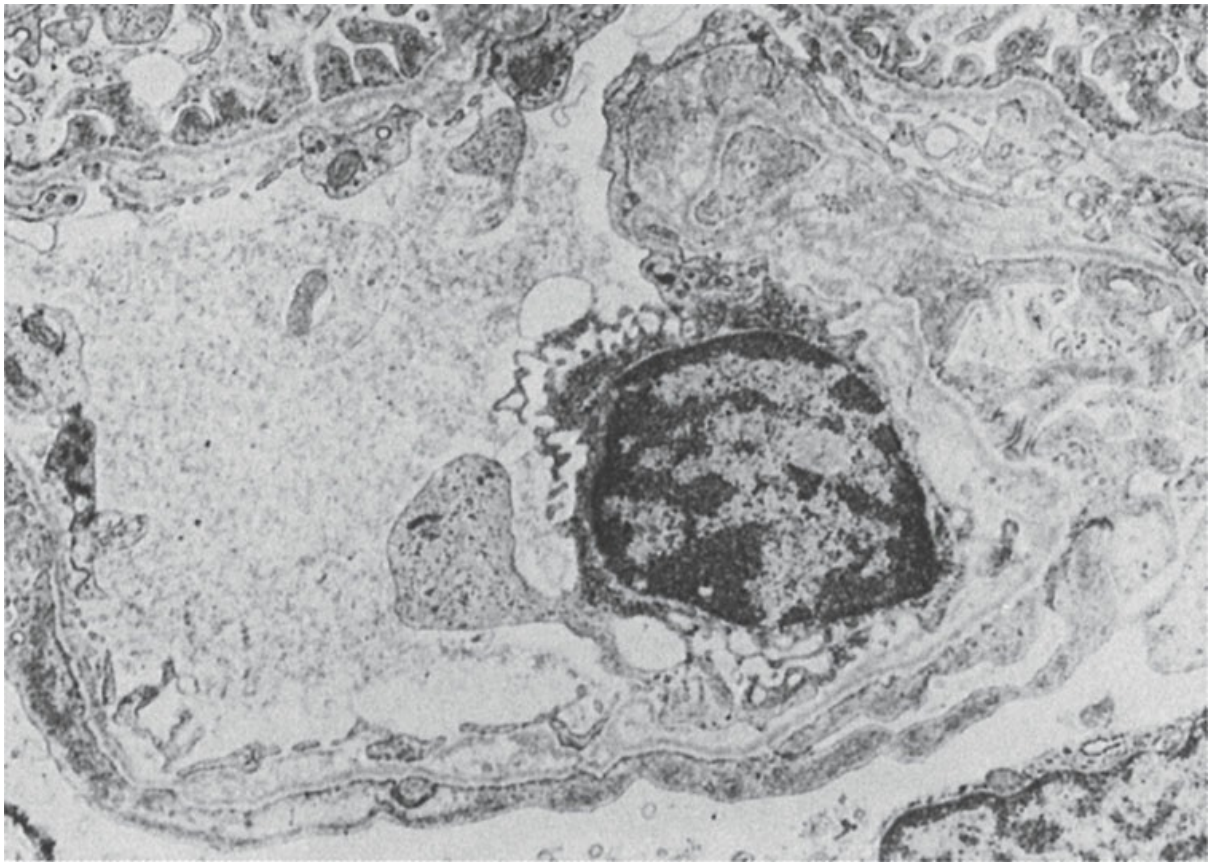

Figure 15–8 Electron microscopic appearance of the lesion in Alport syndrome. Note the thin, altered, glomerular basement membrane.

THIN BASEMENT MEMBRANE NEPHROPATHY

TBMN is an autosomal dominant disorder that frequently results from mutations in two of the genes encoding type IV collagen, *COL4A3* or *COL4A4* on chromosome 2 (286). Its prevalence is estimated to be between 5% and 10% (290). It is frequently familial, with a family history of hematuria reported in almost half of all cases. Patients with TBMN are carriers of autosomal recessive Alport syndrome.

TBMN develops secondary to abnormal collagen that interferes with the normal architecture of GBM. Light and immunofluorescence microscopy are usually normal. EM shows diffuse thinning of the GBM.

TBMD is characterized by persistent or recurrent hematuria that frequently begins in childhood. Proteinuria and hypertension are uncommon. The majority of patients have a benign course and a good prognosis; however, there are reports of secondary focal and segmental glomerulosclerosis developing (290). In patients who develop proteinuria, RAAS inhibitors are recommended.

FABRY DISEASE

Fabry disease is an X-linked lysosomal storage disease caused by a deficiency of α-galactosidase A. The incidence of Fabry disease is estimated at 1 in 40,000 to 1 in 117,000 worldwide (291). There does not seem to be any ethnic predisposition. Usual symptom onset is in childhood and life-threatening complications can develop by middle age in untreated patients. Untreated men have a life expectancy that is 20 years shorter than the general population. Women can develop symptoms but generally at a later age.

Deficiency of α-galactosidase A leads to storage of neutral glycosphingolipids in many tissues. The accumulation of these lipids leads to organ dysfunction. Vascular endothelial cells become enlarged by the storage of glycosphingolipids, which then leads to vascular occlusion and ischemia (291). Symptoms develop in a stepwise fashion usually following an age-specific pattern. Initially, neuropathic pain, ophthalmologic complications, and gastrointestinal symptoms predominate. School difficulties are common. The first renal manifestations are typically proteinuria and isosthenuria that appear in the second or third decade. Most men usually progress to ESRD. The cardiac and cerebrovascular systems are also frequently involved.

Kidney biopsies demonstrate glycolipid accumulation throughout the kidney. Light microscopy shows vacuolization of podocytes and distal tubular epithelial cells. Deposits can be seen later in the parietal epithelial, mesangial, and glomerular endothelial cells. Glomerulosclerosis and tubulointerstitial fibrosis are seen with more advanced disease. Immunofluorescence is nonspecific. EM shows deposits of glycosphingolipids within lysosomes as lamellated membrane structures called myeloid or zebra bodies (Fig. 15-9). These structures are a consistent finding in glycolipid storage diseases (292).

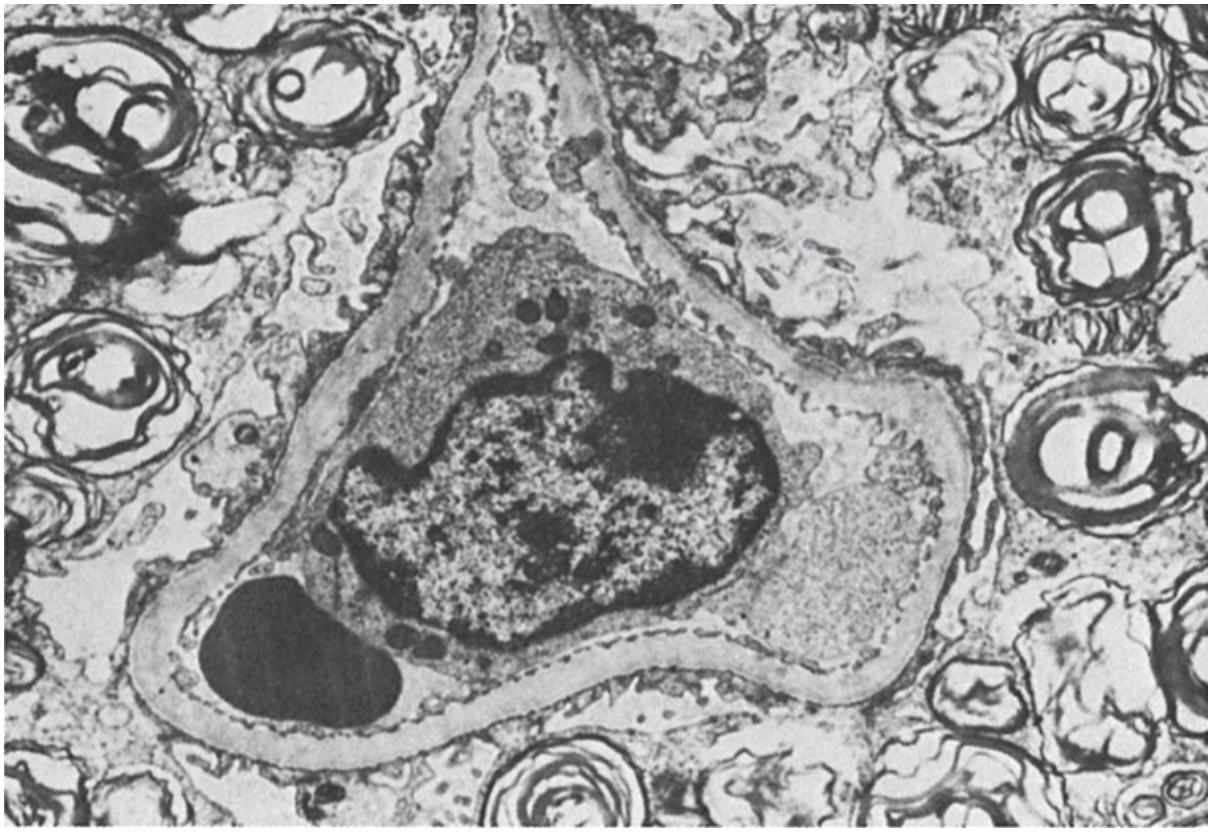

Figure 15–9 Electron microscopic appearance of the lesion in Fabry disease. Note the whorled "myelin" figures in the epithelial cells. (Courtesy of Dr. Arthur H. Cohen.)

Treatment of Fabry disease is enzyme replacement. RAAS inhibitors should be used for proteinuria control.

NAIL-PATELLA SYNDROME

Nail-patella syndrome (NPS) is an autosomal dominant disorder caused by mutations in the gene LMX1B, a transcription factor important for the development of podocytes (293). The incidence of NPS is estimated to be 1 in 50,000. The classic manifestations reflect dysplasia of structures derived from the dorsal mesenchyme, including hypoplastic nails, hypoplastic patellae, elbow dysplasia, and iliac horns. Kidney disease occurs in approximately half of the patients with NPS and is manifested by proteinuria, hematuria, and hypertension. Isothenuria can be seen as well. ESRD develops in approximately 30% of the cases by the third decade (293).

Light microscopy in patients with renal manifestations may show basement membrane thickening and nonspecific lesions of focal and segmental glomerulosclerosis. Immunofluorescence is nonspecific. EM shows irregular thickening of the GBM, including deposits of cross-

banded bundles of striated type III collagen fibers. Foot process may be focally effaced (293).

There is no specific treatment for NPS. RAAS inhibitors have been used to treat proteinuria. Experts also have postulated that cyclosporine may play a role in treatment. NPS does not recur in transplantation (293).

CONGENITAL NEPHROTIC SYNDROME

Nephrotic syndrome that presents at birth or within the first 3 months of life is defined as congenital nephrotic syndrome. Most of the children with congenital nephrotic syndrome have a genetic basis for the renal disease.

A number of different gene mutations have been found as a cause for the majority of cases of congenital nephrotic syndrome. A majority of mutations are of NPHS1 and NPHS2. NPHS1 is the gene responsible for nephrin, an integral part of the slit diaphragm, and is responsible for the Finnish-type congenital nephrotic syndrome. NPHS2 encodes podocin, a protein also important at the slit diaphragm, and is responsible for familial focal glomerulosclerosis (294).

Mutations in several other genes responsible for proteins that are required for proper podocyte function, including α-actinin-4, TRPC6, CD2AP, INF2, and Myo1e, have also been discovered.

Less common mutations are of WT1, which encodes the transcription tumor suppressor and is responsible for the Denys–Drash syndrome, LAMB2 which encodes laminin β-2 and is responsible for the Pierson syndrome, and PLCE1 which encodes phospholipase C epsilon and is responsible for the early onset of isolated diffuse mesangial sclerosis (294).

Congenital nephrotic syndromes are resistant to treatment and have a poor prognosis.

LECITHIN–CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

Lecithin–cholesterol acyltransferase (LCAT) deficiency is an autosomal recessive disorder caused by mutation of the LCAT gene. The enzyme product is responsible for cholesterol esterification. It is characterized by hyperlipidemia, accelerated atherosclerosis, anemia, corneal opacities, and proteinuria. ESRD may result in patients with LCAT deficiency.

COLLAGENOFIBROTIC GLOMERULOPATHY

Collagenofibrotic glomerulopathy, also called collagen type III glomerulopathy, is an autosomal recessive nephropathy characterized by the accumulation of atypical type III collagen fibrils in the mesangium and subendothelial space. Light microscopy reveals findings consistent with MPGN. The fibrils are Congo red negative (nonamyloid) but possess the typical cross striations of mature collagen. A definitive diagnosis requires EM, which reveals fibers with a transverse band structure and a distinctive periodicity of approximately 60 nm (295).

It tends to cause disease in childhood, and may be a familial disorder. Patients often have hypertension, hemolytic anemia, and some patients have had pulmonary disease.

The pathogenesis and treatment are unknown.

LIPOPROTEIN GLOMERULOPATHY

Lipoprotein glomerulopathy is characterized by the deposition of apolipoprotein E (apoE) within glomerular structures (296). Apparently it is the result of mutations in the apoE gene. Nephrotic syndrome and progressive renal failure are common. Theoretically, lipid apheresis could be of benefit, and protein A immunoadsorption was associated with a resolution of the histologic findings and a reduction in proteinuria in an uncontrolled trial (297).

FIBRONECTIN GLOMERULOPATHY

Fibronectin glomerulopathy is an autosomal dominant disorder associated with the deposition of fibronectin. By EM, fibrillar electron-dense deposits are seen in the subendothelial and mesangial spaces (298). Patients may present with proteinuria and microscopic hematuria. Patients with fibronectin glomerulopathy can develop renal insufficiency, and some patients progress to ESRD.

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