

Chapter 42 - Renal and Systemic Manifestations of Glomerular Disease

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PROTEINURIA

Proteinuria characterizes most forms of glomerular injury and causes or contributes to all of the complications of the nephrotic syndrome. This section reviews the physiology and pathophysiology of glomerular permselectivity in clinical and experimental glomerular diseases. Extensive discussion of the mechanisms of proteinuria may also be found in several reviews.^{[1] [2] [3] [4] [5] [6] [7]}

Physiologic Basis of Permselectivity

The glomerular capillary wall (GCW) is extremely permeable to water and small solutes, yet imposes a formidable barrier to the passage of plasma proteins. This permselectivity has been evaluated by characterizing the extent to which the GCW discriminates among molecules of different size, charge, and configuration. Classically, measurement of the Bowman space-to-plasma concentration ratio (the "sieving coefficient," Θ) for various proteins in the rat has been determined by direct sampling via micropuncture techniques.^[1] These studies indicate that small substances appear in the glomerular filtrate in concentrations similar to those in plasma whereas serum albumin is filtered to a much lesser extent (<0.1% that of inulin).

The most extensively used method to quantify glomerular capillary permselectivity involves measurement of the fractional clearance of test macromolecules. For a particular test solute (m), fractional clearance is defined as the urinary clearance of m divided by the glomerular filtration rate (GFR). With the clearance of inulin used to measure GFR, fractional clearance of m is calculated from the urine and plasma concentrations of inulin (I) and m as follows:

$$\text{Fractional clearance of } m = \frac{C_{mu}C_{Ia}}{C_{ma}C_{Iu}}$$

where C refers to solute concentration and the second subscript denotes urine (u) or afferent arteriolar (systemic) plasma (a). If, like inulin, the test macromolecule is not reabsorbed or secreted, its fractional clearance will exactly equal its Bowman space-to-plasma concentration ratio, Θ .^[5]

Proteins are not ideal test markers for such studies because of variations in size, charge, and shape, as well as tubule reabsorption of filtered protein. These difficulties may be circumvented with the use of a variety of exogenous nonprotein polymers, including dextran, dextran sulfate, diethylaminoethyl (DEAE) dextran, polyvinylpyrrolidone, Ficoll, and polyethylene glycol.^[1] Much of the available permselectivity data relate to the use of dextran. However, as discussed later, Ficoll has been evaluated and appears to be superior.^{[6] [8]}

Permselectivity Based on Molecular Size

The use of neutral dextran to analyze glomerular size selectivity is illustrated in the middle curve of [Figure 42-1](#).^{[9] [10] [11]} Measurement of the molecular radii of discrete dextran fractions is based on their elution from gel chromatographic columns calibrated with several proteins of known Stokes-Einstein radius.^[5] A value of 1.0 on the ordinate denotes a dextran clearance equal to that of inulin (e.g., no measurable resistance to the filtration of dextran). Measurable restriction to filtration of neutral dextran does not occur until the effective radius exceeds about 20 Å.

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As dextran size increases, fractional dextran clearance (Θ_D) decreases progressively and approaches zero at dextran radii of at least 40 Å.

Theoretical Interpretation of Size Selectivity

ISOPOROUS MODELS.

The most useful theoretical descriptions of macromolecular transport across the GCW are based on the concept of hindered movement of solutes through water-filled pores.^[12] By using such theoretical analysis, dextran filtration data such as those in [Figure 42-1](#) are accurately predicted by models that envision transport as taking place through numerous, identical cylindrical pores with a radius of approximately 55 Å. Solute molecules are regarded as solid spheres whose movement through pores occurs by diffusion and convection. Fluxes are hindered both by a partitioning phenomenon in which the macromolecule is partially excluded by virtue of its shape, size, or charge and by a hydrodynamic effect related to the nearby presence of the pore wall.^{[6] [13]} For uncharged spherical molecules, interactions between solute and membrane depend on a single parameter, λ , the ratio of solute molecule radius to pore radius. Solute flux declines toward zero as solute size approaches that of the pore, whereas no hindrance is attributable to the pore if the pore is relatively large.

With these concepts in mind, the flux (J_T) of an uncharged solute (T) across the GCW may be expressed in terms of the following: C_T , the concentration of T in glomerular capillary plasma; J_v , the local glomerular transcapillary volume flux; D_T , the diffusivity of T in bulk solution; f , the fraction of the capillary surface area occupied by pores; l , the length of the pores; and hindrance factors that are each unique functions

Figure 42-1 Filtrate-to-plasma concentration ratio (Θ) as a function of molecular size for tritiated dextran sulfate (DS), neutral dextran (D), and diethylaminoethyl dextran (DEAE). Data points are means \pm SE measured in the normal Munich-Wistar rat.^{[9] [10]} All three *solid curves* were calculated theoretically by using the membrane parameters: $K_f = 4.8$ nL/(min \cdot Hg), $r_p = 47$ Å, and $C_m = 165$ mEq/L. (From Deen WM, Satvat B, Jamieson JM: *Theoretical model for glomerular filtration of charged solutes. Am J Physiol* 238:F126, 1980.)

of l . For relatively high fluid flow rates through the pore and for large solutes that diffuse poorly, solute movement is primarily via convection. For lower fluid flow rates or small solutes that diffuse rapidly, or for both, solute movement is governed primarily by diffusion.

The rate of filtration of solute T is dependent on two independent glomerular membrane properties: K_f , the glomerular capillary ultrafiltration coefficient, and r_p , the apparent glomerular pore radius. This term may be evaluated by fitting experimentally observed values of Θ into the model. A more complete discussion of the theories of partitioning and hindered particle motion may be found in several reviews.^{[1] [6] [14] [15]}

Application of this theoretical model to the data in [Figure 42-1](#) results in calculated values of r_p that are relatively independent of molecular size, with an average of about 47 Å. Presumably, all molecules "see" the same pores, so the finding that the "best-fit" value of r_p is independent of molecular size confirms that the theory successfully correlates most of the available data. Values of Θ calculated with the use of the theory for neutral dextran are shown by the middle solid curve in [Figure 42-1](#). In this case, a pore radius of 47 Å provides an excellent fit to the data presented, except for molecular radii smaller than 24 Å, for which the isoporous theory appears to underestimate dextran transport.

An additional parameter that may be derived from values of K_f and r_p is the ratio of total pore area to pore length, fS/l , where f is the fraction of the capillary surface area (S) occupied by pores and l represents pore length. For pores of a given radius and length, this parameter is a measure of "pore density," the apparent number of pores per unit area of the GCW. Assuming that l is roughly the thickness of the glomerular basement membrane (GBM), the dextran data yield an estimate of f to be about 0.1,^[16] thus suggesting that some 10% of the glomerular capillary surface area is perforated by pores.

HETEROPOROUS MODELS.

Theoretical calculations indicate that the normal GCW behaves much as an isoporous filter with a pore radius of about 50 to 55 Å.^{[10] [12]} This approach has proved most useful in

interpreting data for dextrans with effective molecular radii of about 20 to 45 Å. However, in some human diseases, experimental data were found to be incompatible with the isoporous theory. In these proteinuric patients, Θ_D was enhanced for the largest dextrans (>45 Å), but it was often decreased for the smallest dextrans when compared with normal subjects ([Fig. 42-2](#)).^[18] These findings suggested that the selective increase in filtration of large dextrans could be explained by the emergence of a second population of pores, fewer in number but with larger radii, and thus suggested the need for a heteroporous description of functional GCW characteristics. Accordingly, Deen and colleagues^[18] formulated a heteroporous model of glomerular size selectivity designed to account for the experimental observations.

The rationale for this model can be appreciated from a consideration of fractional IgG clearances obtained from subjects with extensive proteinuria.^[18] The large size of IgG ($r = 55$ Å) makes it likely that its passage into the Bowman space is most directly related to the extent of the size-selective defect. As shown in a study of 70 nephrotic patients, considerable variability in IgG excretion was found, with fractional IgG clearances spanning three orders of magnitude, which were used to arbitrarily define three grades of

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Figure 42-2 Fractional dextran clearances (Θ_D) plotted as a function of molecular radius. Grade I (left) and grade III (right) nephrotic patients are compared with normal controls. Values are means \pm SE. (From Deen WM, Bridges CR, Brenner BM, Myers BD: *Heteroporous model of glomerular size selectivity: Application to normal and nephrotic humans. Am J Physiol* 249:F374, 1985.)

increasing barrier injury.^[17] Minimal IgG leakage (grade I) was associated with a low selectivity index (C_{IgG}/C_{alb}), an indication that the GCW was highly permeable to albumin but still relatively impermeable to IgG. At the opposite extreme (grade III), high rates of IgG leakage were associated with a high selectivity index, an indication that the GCW did not discriminate between the smaller albumin and the larger IgG molecule. As is shown in [Figure 42-2](#),^[18] similarities in sieving curves were noted in all grades of injury, with depressed values of Θ_D for smaller and relatively impermeant dextrans and enhanced values of Θ_D for larger macromolecules. However, the curves differed at the point at which the dextran sieving curve intersected that of normal subjects: it occurred earlier with increasing IgG leakage (at $r = 54$ Å in grade I and at $r = 46$ Å in grade III). In addition, the large-radius end of the sieving curve deviated more prominently from normal with increasing injury.

These data are inconsistent with the concept of the GCW as an isoporous filter in that no single population of pores of identical size could simultaneously account for restricted transport of smaller dextrans and enhanced transport of larger dextrans. Rather, the data more closely fit a model of solute transport through a heteroporous membrane with a

subpopulation of large pores. This model assumes that most of the GCW is perforated by cylindrical pores of radius r_0 and that a smaller portion of the GCW is permeated by large, nondiscriminatory "shunt" pathways that do not exhibit size selectivity. The portion of the GCW permeated by shunt pores is denoted Ω_0 , a parameter that quantitates the magnitude of the size selectivity defect. The fractional area of the membrane occupied by this shunt pathway, though small, increases with each successive grade of barrier injury. This subpopulation of large pores is presumed to allow passage of IgG and probably most of the filtered albumin. Therefore, nonselective heavy proteinuria appears to result from loss of barrier size selectivity, which renders the glomerular membrane more porous to large plasma proteins.^[18]

LOGNORMAL MODELS.

In some cases, the isoporous plus shunt model has not accurately fit the data, and better results are obtained with a model that assumes a lognormal distribution of pore radii.^{[8] [19]} This distribution is characterized by u , the mean pore radius, and s , the spread of the distribution.^[19] The model has been further refined by calculation of the theoretical fraction of filtrate volume passing through the largest pores. Remuzzi and associates^[19] used this model to define an index of the size of the largest pores in the GCW. By definition, 5% of the glomerular filtrate passes through pores with radii greater than r^* (5%), and 1% passes through pores with radii greater than r^* (1%).

Although most of the permselectivity data have been obtained with the use of dextran as a marker, a consistent problem has been the finding that Θ for normal subjects tends to be large, given the absence of proteinuria. Thus, dextran appears to overestimate the true Θ . Oliver and co-authors^[8] proposed that Ficoll, which behaves more like an ideal spherical molecule than dextran does, is a better probe of glomerular pore size; the use of Ficoll is now being extended to studies in rats, humans, and in vitro models.^{[20] [21] [22]} In normal rats, Θ for dextran significantly exceeds that for Ficoll at all molecular sizes, being nearly 10 times that of Ficoll for an r_s greater than 30 Å, and values of Θ for Ficoll approximate previously reported values for uncharged glomerular proteins. For Ficoll, a lognormal plus shunt pathway model was found to be the most effective ([Fig. 42-3](#)).^[8]

Permselectivity Based on Molecular Charge

The charge-selective characteristics of the GCW have traditionally been evaluated with negatively charged markers such as dextran sulfate (DS). In a normal kidney, fractional DS clearance (Θ_{DS}) is lower than that for neutral dextran at any given molecular radius (see [Fig. 42-1](#)).^{[5] [11]} Conversely, positively charged molecules pass through more freely (see [Fig. 42-1](#)).^[10] The importance of molecular charge is further demonstrated in proteinuric diseases. In a model of nephrotoxic serum nephritis, Chang and co-workers noted that Θ_D for neutral dextran was less than that seen in normal rats ([Fig. 42-4](#)),^{[5] [10] [23]}^{[24] [25] [26]} and thus these molecules could not account for the observed proteinuria; if albumin behaved like neutral dextran, albumin excretion should decrease rather than increase. Similarly, fractional clearances of cationic DEAE dextran were reduced and therefore could

Figure 42-3 Sieving coefficient (Θ) of dextran and Ficoll as a function of molecular radius (r_s) in normal rats. Experimental values are means \pm SE; curves represent the best fits to the data obtained with the three types of pore size distribution. (From Oliver JD III, Anderson S, Troy JL, et al: *Determination of glomerular size-selectivity in the normal rat with Ficoll. J Am Soc Nephrol 3:214, 1992.*)

not explain the proteinuria. However, for any molecular size, Θ_{DS} in rats with nephrotoxic serum nephritis was substantially greater than that observed in normal animals.^[26] These observations suggested that the albuminuria in this disorder was a specific consequence of the reduction in fixed negative charge of the diseased GCW.

It should be noted that in recent years, the concept of DS as an appropriate marker to assess charge selectivity has been challenged by observations that it is not as inert a tracer as once believed and that earlier studies probably overestimated the effects of charge.^[6] For example, Guasch and colleagues^[27] found that DS binds with plasma proteins. Furthermore, cellular uptake and intracellular desulfation of DS may affect the interpretation of fractional clearance

Figure 42-4 Fractional clearance of neutral dextran (*left*), anionic dextran sulfate (*middle*), and cationic diethylaminoethyl (DEAE) dextran (*right*) plotted as a function of effective molecular radii for normal rats and those with nephrotoxic serum nephritis (NSN). (From Brenner BM, Hostetter TH, Humes HD: *Molecular basis of proteinuria of glomerular origin. N Engl J Med 298:926, 1978.*)

data.^[28] A detailed discussion of controversies in this field may be found in a recent review.^[6] Though not believed to invalidate the concept of charge selectivity, these observations indicate a need for further study in this area.

Permselectivity Based on Molecular Configuration

To compare sieving of molecules with different conformations (i.e., a linear coil for dextran versus lobular proteins), the effects of molecular shape or configuration must be taken into account. Bohrer and associates^[29] compared the fractional clearance of neutral dextran with that of Ficoll, an uncharged cross-linked copolymer of sucrose and epichlorohydrin. At any given effective radius, the flexible coil dextran was filtered more readily than Ficoll, a nearly rigid sphere; the superior accuracy of Ficoll was subsequently confirmed, as described earlier.^[8] These studies suggest that protein configuration also plays a role in filtration, although size and charge appear to be more important.^[1]

ANATOMIC AND FUNCTIONAL BARRIER TO FILTRATION OF MACROMOLECULES.

The classic view of the anatomic barrier to the filtration of protein, as well as the changes that result in pathologic proteinuria, has been extensively reviewed^[2] and will be briefly summarized here. As detailed later, the past few years have seen a major advance in our insight into this process through the discovery of nephrin and associated studies of the molecular nature of the slit diaphragm.

Early studies concluded that the GBM was the component of the GCW that restricted the passage of macromolecules.^[30] Subsequent studies were consistent with this "single-barrier" hypothesis^[2] until examination of the behavior of peroxidative tracers suggested that the slit diaphragm was an effective barrier to filtration.^{[31] [32]} Later studies led to the "double-barrier" hypothesis: that the GBM restricts the passage of larger macromolecules whereas slit diaphragms regulate the passage of smaller ones.^[33] However, this hypothesis failed to explain the findings that some relatively large tracers were restricted just beneath the slit diaphragm and some were completely restricted at the level of the inner layers of the GBM, so the potential contribution of charge needed to be addressed. Rennke and colleagues^[34] used several ferritin fractions of similar size with varying isoelectric

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points (pIs). A stepwise increase in the pI of ferritin resulted in a proportionate increase in its permeation into the GBM, with the more negatively charged particles penetrating furthest. Thus, these studies pointed to the existence of an intrinsic electrical charge in the GBM that was imparted by fixed anionic sites.^[34]

These anionic sites have been localized to the surfaces of endothelial and epithelial cells, as well as the GBM interposed between these cells.^{[2] [35] [36]} The glomerular epithelial cell and its foot processes are covered with a surface coat of acidic glycoproteins (sialoproteins or glomerular polyanion) that are highly negatively charged. Stainable polyanion has been identified to be podocalyxin, a sialoprotein that carries most of the glomerular sialic acid.^[37] The epithelial slit diaphragm also consists, in part, of glycosialoproteins,^[38] as does the endothelial cell coat.

The biochemical composition of the GBM has been extensively studied.^{[2] [39]} The GBM consists of two classes of glycopeptides, a nonpolar collagen-like component and another more polar noncollagen fraction of asparagine-linked polysaccharide units. Glomerular epithelial cells are capable of synthesizing all major GBM components. Integral components of the GBM include type IV collagen, laminin, entactin/nidogen, and various proteoglycans, including chondroitin sulfate proteoglycan and heparan sulfate proteoglycan (HS-PG). Of the latter, HS-PG has been shown to be particularly important in imparting charge selectivity to the GBM.^{[2] [40]} HS-PG is distributed throughout the GBM, but concentrated in the lamina rara interna and externa.^[41] Normally, polyanions (particular HS-PG) act as "antilogging" agents to prevent the adsorption of plasma

protein so that ultrafiltration may proceed.^{[42] [43]} Many studies have indicated the importance of anionic sites and HS-PG specifically in the defense against proteinuria.^{[44] [45]} However, as discussed in the next section, newer evidence points away from a predominant role of the GBM in filtration barrier function.

Newer Concepts in Glomerular Permeability

Tracer studies as described earlier cannot differentiate the effects of hemodynamic and hormonal influences from the properties intrinsic to glomerular filtration. Innovative models have been developed to assess glomerular permeability in vitro. Daniels and co-workers^{[22] [46] [47] [48]} examined the diffusion of fluorescent macromolecules across individual glomerular capillaries in intact glomeruli by confocal microscopy. With this method, the relative contribution of glomerular cells may be assessed by comparing intact with acellular glomeruli from which cells have been removed and leaving the GBM with some residual mesangial matrix. Studies in this system showed that Θ_D in intact glomeruli is much less than that for the GBM alone; most of the size and charge selectivity of the GCW resides in the cells rather than in the GBM. In vivo studies have provided further confirmation of the importance of podocytes^[49] and slit diaphragms^{[6] [50]} in the restrictive properties of the GCW.

Traditional observations of changes in the structure of glomerular epithelial cells in various clinical proteinuric diseases prompted speculation that defects in that region might be responsible for increased GCW permeability.^[51] More recently, alterations in several epithelial cell proteins, including megalin,^[52] glomerular epithelial protein-1 (GLEPP-1),^[53] the Wilms tumor gene protein (WT-1),^[54] and nephrin,^[55] have been associated with nephrotic syndromes. Nephrin, which localizes to the slit diaphragm of visceral epithelial cells, is mutated in a severe form of congenital nephrotic syndrome (*NPHS1*).^[55] Newer candidate genes identified as potentially being associated with nephrotic syndromes now include *Mpv 17*,^[56] *NPHS2*,^[57] and *NEPH1*.^[58]

Influence of Hemodynamic Factors on Filtration of Macromolecules: General Considerations

Hemodynamic factors influence the filtration of macromolecules. Often, Θ varies inversely with the single-nephron GFR (SNGFR).^{[11] [11] [59]} Thus, filtration of macromolecules is influenced not only by the intrinsic membrane properties of the GCW but also by other determinants of SNGFR: Q_A , the glomerular capillary plasma flow rate; ΔP , the glomerular transcapillary hydraulic pressure difference; and C_A , the afferent arteriolar plasma protein concentration. The absolute single-nephron clearance of a macromolecule is given by the product $\Theta \times \text{SNGFR}$.^{[11] [15]} Absolute clearance usually increases as Q_A is elevated, but less than in proportion to SNGFR; hence, Θ decreases. The effect of Q_A is explained by considering that at a high flow rate, a given amount of filtrate produces a smaller increase in the plasma concentration of the test solute, thus making the increased filtration of neutral molecules less than that of water and thereby decreasing Θ . Absolute macromolecular clearance rates also increase as ΔP rises. For neutral and anionic macromolecules, this increase is less than the increase in SNGFR,

and as a result, Θ decreases. For highly anionic molecules, this trend reverses at sufficiently high ΔP , and Θ may increase. The opposite behavior is observed for positively charged macromolecules, with Θ increasing with rising ΔP . The theoretical effects of C_A on Θ are similar to those for inverse changes in ΔP because C_A and ΔP exert opposing effects on SNGFR. The actual effects of changes in C_A are likely to be more complicated because of parallel changes in K_f .^[60] Hemodynamic factors may also influence rates of volume flux through the shunt pathway (see later).

Evaluation of the Filtration Barrier in Glomerular Disease

Animal Models of Glomerular Disease

Tracer macromolecules have been used in a number of experimental proteinuric models to characterize the glomerular permeability defects that result in proteinuria, as well as the response to therapeutic interventions. Not surprisingly, all such models exhibit impaired glomerular size selectivity with the passage of large molecules through the glomerular barrier. This defect has been noted in diverse animal models, including renal ablation,^{[61] [62] [63]} diabetes,^[20] and many others.^[64] In some models, a charge-selective defect has also been seen. For example, a reduction in renal mass leads to hypertension and proteinuria, with elevated SNGFR, Q_A , and ΔP and reduced K_f . In this model, the proteinuria is due to defects in both size and charge selectivity,^{[61] [62] [63]} with resultant increased flux through the shunt pathway. Peak pore size (u) is decreased whereas the width of the pore size distribution (s) is increased; both $r^*(5\%)$ and $r^*(1\%)$ increase

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Figure 42-5 Fractional clearance of Ficoll as a function of molecular radius (\AA) in diabetic and nondiabetic control rats (*left*) and in diabetic rats treated with losartan (*right*). Values are means \pm SE. * $P < .05$; ** $P < .01$ versus controls; ° $P < .05$; °° $P < .01$ versus the diabetic group. (From Remuzzi A, Perico N, Amuchastegui CS, et al: Short- and long-term effect of angiotensin II receptor blockade in rats with experimental diabetes. *J Am Soc Nephrol* 4:40, 1993.)

as well.^[63] Another prominent example is diabetic nephropathy, a model with similar hemodynamic changes (see [Chapter 38](#)). Studies using Ficoll in diabetic rats found impaired size selectivity at all molecular radii tested ([Fig. 42-5](#)); in addition, the pore size distribution was shifted toward larger radii, as reflected by increases in u , w_0 , and $r^*(1\%)$.^[20]

Another model is produced by injecting puromycin aminonucleoside into rats, which induces massive proteinuria and predominant epithelial cell injury. Ultrastructural studies indicate focal permeability to tracer macromolecules, particularly in areas of epithelial detachment from the GBM,^{[33] [65]} along with altered distribution of anionic sites and sulfation of heparan sulfate.^[44] In a puromycin aminonucleoside regimen that results in diminution of K_f with relative preservation of ΔP , Q_A , and K_f , Θ_D for neutral dextrans 38 Å and smaller is decreased, whereas Θ_{DS} is enhanced. Because glomerular hemodynamics is normal in this model, these data suggest a functional loss of the electrostatic barrier.^[66] The Θ_D of neutral dextrans larger than 38 Å is also increased, thus suggesting that a size-selective defect likewise contributes to proteinuria in this model.^[67] Injection of neutral dextran into rats with doxorubicin (Adriamycin) nephrosis results in increased clearance of molecules larger than 40 Å.^{[19] [68]} The lognormal model indicates increases in u and s , as well as in $r^*(1\%)$ and $r^*(5\%)$.^[19] The role of a charge-selective defect is controversial.^[19]^[68] Studies in other models have been reviewed recently.^[64]

Effects of Hemodynamic Changes on Glomerular Permselectivity

The influence of hemodynamic changes on glomerular permselectivity has also been widely studied. Acute hypertension induced by angiotensin II (AII) enhances protein filtration,^[9] and AII also plays a role in the model of partial renal vein constriction.^[69] This maneuver led to a marked decrease in Q_A with a lesser decrease in SNGFR, an increase in ΔP , and an increase in Θ_D for dextrans larger than 44 Å. The AII receptor antagonist saralasin normalized glomerular hemodynamics. Fractional clearance of dextrans larger than 46 Å fell toward normal, whereas clearance of dextrans smaller than 40 Å was unaffected. The radius of the small selective pores and the index for fractional volume flux through these pores were relatively unaffected. However, renal vein constriction induced a 10-fold increase in the fraction of filtrate passing through larger, nonselective pores. About half the increase was reversed with saralasin. Thus, these data suggested that AII enhances proteinuria by inducing reversible changes in the sieving properties of the GCW, specifically by increasing flow through the shunt pathway, and that these changes might relate to perturbations in ΔP .^[69] The action of AII is somewhat variable, however. Clinical studies have failed to find such an effect of AII in nephrotic subjects, whereas AII did increase fractional neutral dextran clearance in normal subjects; however, the effect could be attributed solely to hemodynamic factors.^[70]

Human Glomerular Disease

PROTEIN CLEARANCE AND SELECTIVITY INDEX.

Nephrotic-range proteinuria is associated with the passage of large plasma proteins into urine. The permselective characteristics of such glomeruli have been determined by measurement of the urinary clearance of proteins of graded size.^[71] The smallest proteins, usually albumin (36 Å) or transferrin (38 Å), have been used as reference markers. When the clearance ratio of larger test proteins to that of the reference protein is plotted as a function of molecular weight, an inverse relationship is seen, which suggests that the diseased glomerulus continues to discriminate among proteins of increasing size but that

the pore size distribution is shifted to pores of larger size. Thus, two categories of clinical proteinuria have been designated "selective" and "nonselective." Selective proteinuria is characterized by a relatively sharp molecular size cutoff; the proteinuria consists primarily of relatively small albumin molecules. Nonselective proteinuria contains a large proportion of larger plasma proteins, particularly IgG. When the selectivity index (the clearance ratio of a large protein such as IgG to that of a smaller protein such as albumin) is less than 0.2, the proteinuria is considered to be selective, whereas values greater than 0.2 indicate nonselective proteinuria.

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This method, though relatively simple, has some theoretical limitations. The test proteins used have different pIs in physiologic solution, and the technique is unable to take into account changes in glomerular charge selectivity. Similarly, the technique cannot account for changes in tubular reabsorption of proteins, the protein catabolic capacity, or a potential selective increase in the glomerular filtration of one or more proteins. In view of these limitations, dextran (and, more recently, Ficoll) clearance techniques have been used to characterize clinical alterations in glomerular permselectivity.

STUDIES OF GLOMERULAR BARRIER FUNCTION IN CLINICAL GLOMERULAR DISEASE.

The defective permselectivity in various forms of clinical proliferative glomerulonephritis was examined by determining the fractional clearance of dextran and IgG.^[22] The Θ_b values of smaller dextrans were similar regardless of the magnitude of IgG excretion, whereas those of larger dextrans were elevated in patients excreting larger amounts of IgG. These data could be best explained by the existence of a second population of larger pores (i.e., the shunt pathway). This model predicted that the passage of macromolecules such as IgG may be totally unrestricted in the damaged segment, and in fact, the filtered load of IgG was sufficiently large to account for the urinary IgG content. Thus, it was conceivable that IgG passage was entirely through the larger pores whereas passage of the smaller albumin molecule was more likely due to a charge-selective defect in the small-pore component of the GCW. The estimated radius of small pores was similar in the two groups, but the radius of larger pores was increased in patients with greater urinary loss of IgG.^[22] These data suggested that when glomerulonephritis is associated with selective proteinuria, the major abnormality is in the charge-selective barrier to smaller proteins, whereas in glomerulonephritis associated with nonselective proteinuria and massive IgG loss, the GCW exhibits a subpopulation of enlarged pores that are highly permeable to proteins of large size and variable charge.

Subsequent studies have found that in many human glomerular diseases, the permselectivity defect consists of a combination of impaired size and charge selectivity and increased volume through the shunt pathway. To cite just a few of many examples, this pattern has been suggested in patients with minimal change disease,^{[17] [22] [23]} membranous nephropathy,^{[21] [74] [75] [76]} and diabetic nephropathy.^{[77] [78] [79]} In diabetes, the

dextran clearance profile changes in the evolutionary stages of diabetic nephropathy.^{[79] [80] [81] [82] [83] [84] [85] [86] [87]} In patients with microalbuminuria (<300 mg/day), studies using IgG^[80] and neutral dextrans^[81] confirm the presence of a size-selective defect. Filtration of dextrans smaller than 48 Å is increased and that of larger dextrans is enhanced with increased filtrate volume through the shunt pathway.^[81] Studies in subjects with overt nephropathy indicate qualitatively similar changes, though enhanced in magnitude.^{[80] [81]} The data are also most consistent with the presence of a concomitant charge-selective defect. Indeed, the charge defect may even precede the size defect.^[82] In diabetic patients, glycosylation of proteins may contribute to the problem inasmuch as glycosylated proteins, including albumin, appear in urine more readily than nonglycosylated forms do^[83] and nonenzymatic glycation of albumin increases its permeability through the GBM in vitro.^[84] In addition, it is possible that shape changes contribute to diabetic proteinuria and that the density of sites that attract polyanions may be increased in diabetic patients.^[85] The present data suggest that the primary abnormality in diabetes is a size-selective defect, but that charge and perhaps shape selectivity defects also contribute.^{[77] [81]}

Interventions and Modulation of Permselectivity

Many pathophysiologic and therapeutic interventions that are known to influence proteinuria have been explored by using the aforementioned techniques. Most of the studies have assessed size, but not charge permselectivity. Interventions such as plasma volume (PV) expansion,^{[5] [86]} dietary protein restriction,^{[19] [61] [87]} and others^[64] restore normal glomerular size selectivity. Of note, although some antihypertensive regimens tend to reverse size-selective defects, others do not. Moreover, interventions that successfully restore size selectivity do not do so in a uniform manner. Although many interventions reduce permeability only for larger dextrans, drugs that block AII formation (angiotensin-converting enzyme [ACE] inhibitors and the AII receptor antagonist losartan) appear in some cases to reduce the clearance of neutral dextrans of all sizes^{[20] [88] [89]} (as shown in [Fig. 42-5](#)).^[20] In other cases, however, the same interventions were found to reduce the clearance of only the largest macromolecules.^{[63] [90]} The ability of ACE inhibition to improve size selectivity has been shown in type 1 diabetic patients,^{[91] [92]} but it may be less consistent in type 2 diabetes.^[93]

Clinical Consequences of Proteinuria

Loss of albumin and other proteins into urine is the hallmark of nephrotic syndrome and a proximate or contributing cause to virtually all the systemic complications of this disorder. As depicted in [Figure 42-6](#)^[94] and detailed later, increased filtration of plasma proteins contributes to hypoalbuminemia and its complications, to hyperlipidemia, to alterations in coagulation factors, and to alterations in cellular immunity, hormonal status, and mineral and electrolyte metabolism (for reviews, see references ^{[94] [95] [96] [97] [98]}).

HYPOALBUMINEMIA

Pathogenesis of Hypoalbuminemia

The hypoalbuminemia of nephrotic syndrome results from multiple abnormalities in albumin homeostasis and is only partially explained by urinary albumin loss.^{[3] [94] [95] [96] [97] [98]} Normal albumin metabolism is schematized in the upper panel of [Figure 42-7](#).^[98] The liver normally synthesizes 12 to 14 g/day of albumin, 90% of which is catabolized in extrarenal sites, primarily the vascular endothelium.^{[100] [101]} About 10% of the albumin synthesized daily is catabolized in the kidney, mainly by proximal tubule reabsorption of filtered albumin.^[102] About 150 g of albumin (or 30% to 50% of the total exchangeable pool) is located intravascularly, with the remainder in interstitial fluid, mostly skin and muscle.^[103] The fractional catabolic rate, or the percentage of the plasma pool that is catabolized daily, is about 6% to 10%.^{[100] [104] [105]} Thus, nephrotic hypoalbuminemia could result from some combination of urinary loss, decreased or insufficiently

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Figure 42-6 Pathophysiology of nephrotic syndrome. All abnormalities originate from increased glomerular permeability to plasma proteins; hypoalbuminemia initiates the major manifestations. (From Bernard DB: *Extrarenal complications of the nephrotic syndrome. Kidney Int* 33:1184, 1988.)

Figure 42-7 Daily albumin turnover in normal individuals (A) and in patients with nephrotic syndrome (B). (Reproduced from Bernard DB: *Metabolic complications in nephrotic syndrome: Pathophysiology and complications. In Brenner BM, Stein JH [eds]: The Nephrotic Syndrome, Vol 9. New York, Churchill Livingstone, 1982.*)

increased hepatic albumin synthesis, increased albumin catabolism, or altered albumin distribution.^{[3] [99]}

EXTRACORPOREAL LOSSES.

The magnitude of hypoalbuminemia tends to increase with increasing proteinuria, but significant hypoalbuminemia can occur in the presence of urinary loss of only a few grams of albumin per day. Urinary losses alone should not necessarily lead to hypoalbuminemia because the liver can easily augment albumin synthesis and thus compensate for such losses.^[101] Evidence for enhanced intestinal albumin loss, or increased albumin catabolism, in the nephrotic syndrome is inconsistent and not compelling.^[99] As discussed later, renal albumin catabolism is increased, thereby contributing to the greater tendency to hypoalbuminemia.

HEPATIC ALBUMIN SYNTHESIS.

Hepatic albumin synthesis is not impaired and, in fact, may be increased by as much as 300% in the nephrotic syndrome.^{[106] [107] [108]} In nephrotic rats, hepatic release of albumin is enhanced,^[109]

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and the relative synthetic rate of albumin is markedly increased, with a comparable increase in albumin mRNA. The relative amounts of several other mRNA molecules, including those encoding for β -fibrinogen, haptoglobin, and metallothionein II, are increased, whereas the amount of mRNA encoding for α_1 -acid glycoprotein is decreased.^{[109] [110]} Oncotic pressure may play a role in albumin synthesis in as much as albumin gene expression varies inversely with oncotic pressure in experimental models.^[111] That a transcriptional process is mainly responsible is suggested by findings that both steady-state levels and transcription rates of albumin mRNA are increased in the livers of nephrotic rats.^{[112] [113]} However, the increase in hepatic albumin synthesis is not maximal and is inadequate for the degree of hypoalbuminemia; thus, the albumin synthetic response rate is relatively impaired.

ALBUMIN CATABOLISM.

In some hypoalbuminemic states, albumin catabolic rates are reduced.^[114] In contrast, the possibility that hypoalbuminemia might be exacerbated by a maladaptive increase in albumin catabolism was suggested by Katz and co-authors,^{[115] [116]} who speculated that the increased urinary albumin load might up-regulate tubular albumin catabolism. In that case, most filtered albumin would be catabolized, and thus urinary albumin would represent only a small fraction of the filtered load. In confirmation of this notion, tubule albumin reabsorptive rates increase in rats with nephrotoxic serum nephritis, though variably.^[117] Nephrotic rats have protein reabsorption droplets containing both albumin and globulin in the proximal and distal tubule cells,^[118] and tubular lysosomal activity increases with an increased urinary protein load.^[119] Additional support for the concept comes from evidence of a dual transport system for albumin uptake in the isolated perfused rabbit proximal tubule. This model exhibits both a low-capacity system that becomes saturated once the protein load exceeds physiologic levels and a high-capacity, low-affinity system that permits tubule albumin reabsorptive rates to rise as the filtered load increases.^[120] Thus, an increase in the fractional catabolic rate may occur in the nephrotic syndrome.

Micropuncture studies indicate that albumin reabsorption may, in fact, be saturated at near-physiologic levels, so most of the urinary albumin is excreted rather than catabolized,^{[117] [121] [122]} and the albuminuria does not markedly underestimate overall albumin loss. Regardless of whether fractional catabolism is normal or increased, total body albumin stores are markedly decreased in the nephrotic syndrome. The net result is that absolute catabolic rates are normal or decreased.^{[106] [115] [123] [124]} As discussed later, nutritional considerations affect this process. In nephrotic rats, absolute catabolic rates are decreased in rats fed adequate dietary protein but increased in rats receiving a low-

protein diet.^[123] Although decreased catabolism may serve to preserve total albumin stores in the face of massive albuminuria, it is obviously insufficient to maintain albumin homeostasis.

ALBUMIN DISTRIBUTION.

In nephrotic syndrome, the extravascular albumin pool is even more depleted than the intravascular pool,^{[125] [126]} the mechanisms of which are discussed later. However, although mobilization of extravascular albumin represents an early response to acute albumin loss, this compensatory mechanism is clearly inadequate in the setting of continuing albumin loss, as in nephrotic syndrome.

Regulation of Albumin Metabolism in Nephrotic Syndrome

Several factors contribute to regulation of albumin metabolism and probably contribute to dysregulation in nephrotic syndrome.^{[99] [124]} The most widely studied factors regulating albumin synthesis are serum oncotic pressure and nutritional status (particularly dietary protein intake).

Whereas albumin synthesis in an isolated perfused liver preparation is inversely proportional to the oncotic pressure of the bathing solution,^[127] albumin synthetic rates do not correspond to either serum albumin concentration or oncotic pressure in nephrotic patients.^{[107] [115]} It has been postulated that the hepatic albumin synthetic rate is more directly determined by changes in the hepatic extravascular interstitial albumin pool than by plasma characteristics and that this hepatic pool is not depleted in nephrotic syndrome and thus albumin synthesis is not stimulated.^{[127] [128]} More recently, it has been suggested that some serum factor or factors in hypo-oncotic states may stimulate albumin synthesis. In support of this hypothesis, incubation of rat hepatocytes with serum from nephrotic rats led to increased albumin and transferrin synthesis, even when oncotic pressure in the medium was normalized.^[129]

Dietary factors also play a role. Kaysen and associates^[113] found that albumin synthesis and serum albumin were not correlated in nephrotic rats fed a low-protein diet but that in the presence of high protein intake, albumin synthetic rates varied inversely with serum albumin. Increasing dietary protein intake in nephrotic rats results in increased hepatic albumin mRNA content, as well as increased transcription, whereas decreased dietary protein intake limits hepatic albumin synthesis.^{[113] [130]} Hepatic albumin synthesis may also respond to changes in dietary fat intake,^[131] as well as to the relative proportion of protein to nonprotein calories.

These observations suggest that in nephrotic syndrome, the optimal diet would include adequate caloric intake with a moderate- to high-protein diet. However, increasing dietary protein intake does not increase serum albumin or body albumin pools in nephrotic animals^{[123] [132]} or patients.^[123] As depicted in [Figure 42-8](#),^[123] feeding a high-protein diet markedly stimulates hepatic albumin synthesis in nephrotic rats.^{[123] [130]} This beneficial effect does not correct hypoalbuminemia, however, because dietary protein

supplementation also increases urinary protein loss. This unfortunate consequence of a high-protein diet also occurs in nephrotic patients; those eating a high-protein diet exhibit higher albumin synthetic rates, but also increased albuminuria, which results in no change in serum albumin levels.^[123]

Factors contributing to enhanced proteinuria in the setting of a high-protein diet may include increased renal blood flow and GFR, with enhanced fractional renal clearance of albumin,^[133] and stimulation of the renin-angiotensin system.^[134] The exact dietary component of protein that stimulates albumin synthesis is unknown but does not appear to be either arginine^[135] or branched-chain amino acids.^[136] However, the net result is that despite the enhanced albumin synthesis, increased urinary losses predominate, so the serum albumin concentration and body albumin pools are further reduced.^[137] Experimentally, blockade of the renin-angiotensin system in the setting of a high-protein diet

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Figure 42-8 Relationship between albumin synthesis, catabolism, and albuminuria in nephrotic rats fed 21% or 40% protein diets. (From Kaysen GA, Kirkpatrick WG, Couser WG: *Albumin homeostasis in the nephrotic rat: Nutritional considerations*. *Am J Physiol* 247:F192, 1984.)

allows increased hepatic synthesis but limits proteinuria, thereby allowing some amelioration of the hypoalbuminemia.^[130] ^[132] In nephrotic patients, both dietary protein restriction and ACE inhibition reduce proteinuria; however, protein restriction also reduces hepatic albumin synthesis, whereas albumin synthetic rates are maintained with ACE inhibition.^[137]

Hormones such as insulin,^[138] thyroid hormone,^[139] growth hormone,^[140] and glucocorticoids^[141] are all needed for albumin synthesis, but their relevance to nephrotic hypoalbuminemia is not well understood. Albumin synthesis is suppressed in the presence of inflammation,^[142] and it is possible that elevated levels of lymphokines such as tumor necrosis factor ^[143] interfere with albumin synthesis in nephrotic syndrome.

In summary, nephrotic hypoalbuminemia is characterized by large urinary albumin losses and a marked reduction in the total exchangeable albumin pool. Mechanisms tending to counteract these forces are mobilization of extravascular pools, increases in albumin synthesis, and decreased albumin catabolism. However, these compensatory mechanisms are insufficient to correct the hypoalbuminemia. Comparisons between normal and nephrotic albumin homeostasis are schematized in the bottom panel of [Figure 42-7](#).^[98] Normally, hepatic synthesis equals the amount catabolized, with a yield of 1 to 2 g, which undergoes glomerular filtration and proximal tubular catabolism. In the nephrotic state, hepatic synthesis may be slightly increased, but the plasma albumin pool is smaller because catabolism is proportionally enhanced. Larger amounts are presented to the

glomerulus, thereby resulting in both increased urinary loss and enhanced tubule catabolism.

Consequences of Hypoalbuminemia

Hypoalbuminemia causes or exacerbates numerous complications of the nephrotic syndrome, including abnormalities in blood volume and composition, edema formation, impaired renal function, increased platelet aggregability, enhanced potential for drug toxicity, and hyperlipidemia.

Edema Formation and Blood Volume Homeostasis

Nephrotic edema does not result solely from hypoalbuminemia. Transcapillary fluid flux (J_v) across a membrane is defined by the Starling relationship: $J_v = L_p (\Delta P - \Delta \Pi)$, where ΔP and $\Delta \Pi$ are the transmembrane hydrostatic and oncotic gradients, respectively; L_p is the hydraulic conductivity of the membrane; and s is the reflection coefficient for plasma proteins, mainly albumin.^{[144] [145] [146]} The balance of Starling forces at the arteriolar end of the capillary ($\Delta P > \Delta \Pi$) favors net filtration of fluid into the interstitium.^[144] However, ongoing fluid transudation (edema accumulation) is normally limited by at least three protective mechanisms. First, the lymphatics expand and proliferate so that increased lymphatic flow provides protection. Second, transudation of protein-free filtration into the interstitium reduces interstitial oncotic pressure (Π_{IF}), thus decreasing ΔP and slowing ultrafiltration. Third, fluid flux tends to increase interstitial hydrostatic pressure (Π_{IF}), thereby reducing ΔP and further slowing filtration.^[147]

Furthermore, the compliance characteristics of the interstitium resist fluid accumulation.^{[148] [149]} Compartmentalization within the interstitial space prevents rapid local translocation of fluid in subcutaneous and subserosal tissue. Thus, the appearance of edema in glomerulonephritis implies substantial disruption of the normal defenses against edema formation.^[145] Generalized edema therefore implies substantial and ongoing renal Na^+ retention, further supporting the concept that intrarenal mechanisms prevail in the pathogenesis of the Na^+ retention associated with glomerular disease (see later).^{[150] [151] [152]}

RELATIONSHIP OF EDEMA FORMATION TO REDUCED PLASMA ONCOTIC PRESSURE.

According to the traditional view of nephrotic edema formation, hypoalbuminemia lowers the colloid oncotic pressure of blood, thereby favoring movement of water from the vascular to the interstitial space. However, continued edema formation would require disruption of normal defenses against edema, and evidence for such derangement is not clearly found. For example, in nephrotic patients, hypoalbuminemia is

accompanied by a fall in Π_{IF} sufficient to substantially retard interstitial fluid accumulation.^[153] Values of Π_{IF} in nephrotic animals and patients also fall virtually in parallel with the decrease in plasma colloid osmotic pressure and serum albumin levels, thus maintaining net transcapillary ΔP in the normal range.^{[154] [155] [156]} Patients studied during relapse and remission show almost equivalent changes in interstitial and plasma colloid osmotic pressure.^[156] The reduction in Π_{IF} results in part from acceleration of lymphatic flow, which in turn returns interstitial protein to the intravascular space.^{[156] [157] [158]} It has been suggested that this "wash-down" phenomenon is triggered by a slight increase in interstitial volume and hydraulic pressure induced by the initial loss of fluid into the interstitium. Body albumin pools are thus redistributed so that a greater fraction is located in the intravascular space; the interstitial albumin concentration may be as low as 5% of that in plasma in nephrotic patients.^[125] These events thus serve to maintain blood volume and defend against edema formation. ^{[3] [98]}

Accordingly, it appears that substantial disruption of the renal mechanisms responsible for extracellular fluid homeostasis, rather than the level of hypoalbuminemia per se, is the primary determinant of the severity of edema formation. In assessing the relative contribution of hypoalbuminemia to edema formation, it is necessary to take into consideration the prevailing intravascular volume as well.

RELATIONSHIP OF EDEMA FORMATION TO THE PREVAILING INTRAVASCULAR VOLUME.

One postulated scenario linking hypoalbuminemia to edema formation relates to "underfill mechanisms," as depicted in [Figure 42-9](#).^{[152] [159] [160] [161] [162] [163]} According to this scenario, reductions

Figure 42-9 The "underfill" mechanism of edema formation. Hypovolemia (resulting from hypoalbuminemia and decreased plasma oncotic pressure) is viewed as the key event promoting Na^+ and water retention by the kidney. (From Perico N, Remuzzi G: *Edema of the nephrotic syndrome: The role of the atrial peptide system. Am J Kidney Dis* 22:355, 1993.)

in serum albumin, and therefore plasma oncotic pressure, lead to edema formation, but also to hypovolemia. The reduced PV then triggers compensatory mechanisms (e.g., nonosmotic vasopressin release, the renin-angiotensin-aldosterone system, and the sympathetic nervous system) that stimulate renal Na^+ and water retention. The latter serve to restore intravascular volume but also exacerbate hypoalbuminemia, so edema formation continues. However, some experimental observations are at odds with this hypothesis.^{[95] [145] [159] [161]} First, the clinical syndrome of congenital analbuminemia is not necessarily associated with edema,^[164] nor is the transcapillary $\Delta\Pi$ abnormal in analbuminemic rats.^[165] Second, the presence of hypovolemia is questionable. The evidence against hypovolemia as the proximate cause of Na^+ retention is threefold: an inability to document hypovolemia by direct measurements, an inability to consistently find

changes in hormonal modulators compatible with hypovolemia, and failure of predicted changes to occur after remission or diuretic therapy. In nephrotic patients, PV and blood volume are not usually reduced; in fact, they are generally normal or even expanded. ^{[166] [167] [168] [169] [170] [171] [172] [173] [174] [175]}

Many of the available studies actually note a range of PV in patients studied. Moreover, methodologic differences and limitations in blood and PV measurements may interfere with the interpretation of these studies. ^{[152] [162] [168]} Nonetheless, it should be possible to indirectly estimate blood volume by measurement of vasoactive hormones that change in response to altered intravascular volume. Thus, reduced intravascular volume should be reflected by elevated values for hematocrit and plasma renin activity (PRA), aldosterone, arginine vasopressin (AVP), and norepinephrine concentrations and reduced values for atrial natriuretic peptide (ANP). Such functional evidence of hypovolemia is not consistently found in nephrotic syndrome. ^{[98] [152] [159] [161] [168]} PRA and aldosterone levels tend to be reduced and are not always well correlated with changes in blood volume. ^{[166] [167] [169] [171]} Similarly, plasma levels of norepinephrine, AVP, and ANP tend to be normal (or inconsistently changed). ^{[168] [170] [176] [177]} Moreover, PV expansion by infusion of hyperoncotic plasma ^[178] or salt-poor albumin ^[179] and head-out water immersion ^{[180] [181]} does not regularly result in diuresis or natriuresis. However, despite the lack of a uniform response, some studies find evidence consistent with hypovolemia and a natriuretic response to these maneuvers. ^{[152] [162] [178] [182]}

Evidence from patients undergoing remission from nephrotic syndrome is likewise unclear. In responsive patients, steroid therapy leads to diuresis and natriuresis before any change in serum albumin. PRA and aldosterone levels are initially high and fall during natriuresis. After resolution of the edema, PRA and aldosterone again rise to high levels, whereas plasma albumin and blood volume remain low; however, Na⁺ retention does not occur, and Na⁺ balance is maintained. ^[169] Thus, the absence of Na⁺ retention in the setting of evidence of hypovolemia and hypoalbuminemia during remission points to an intrarenal defect as the probable cause of edema during the nephrotic syndrome. Resolution of this intrarenal defect is characterized by an increase in the filtration fraction, again suggesting that natriuresis results from renal repair rather than changes in blood volume. ^[183]

Taken together, these observations suggest a wide spectrum in prevailing PVs. Indeed, one study found a suggestion

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of two populations of patients. Those with steroid-responsive minimal change disease tended to have volume contraction and high PRA values, whereas patients with more advanced steroid-resistant disease exhibited PV expansion and suppression of the renin axis. ^[166] In head-out water immersion studies, the natriuretic response was correlated with the pre-immersion state of Na⁺ balance. ^[181] Further evidence comes from studies of nephrotic rats in the

presence or absence of PV expansion induced by a reduction in renal mass.^[184] Rats with chronic renal failure exhibited PV expansion that progressed when they became nephrotic; edema formation did not occur in the presence of chronic renal failure or nephrotic syndrome alone, only when nephrotic rats were PV expanded.^[184] These data have important therapeutic implications. The evidence suggests that edema is not necessary for maintenance of blood volume and, as a corollary, that vigorous treatment of edema with diuretics does not cause failure to maintain blood volume.^{[179] [185]}

ROLE OF INTRARENAL MECHANISMS.

No single mechanism accounts for edema formation in all nephrotic patients. However, changes in blood volume are not solely sufficient to explain the avid Na^+ retention that occurs clinically. Most of the available evidence implicates a primary intrarenal defect in this disorder. This hypothesis, termed the "overflow theory," is schematized in [Figure 42-10](#).^[152] According to this hypothesis, a primary increase in renal Na^+ retention leads to extracellular fluid volume expansion, altered Starling forces, and edema formation. Evidence in support of this mechanism comes from observations that Na^+ retention occurs only in the ipsilateral kidney of dogs^[186] and rats^{[150] [187]} with unilateral glomerulonephritis. Micropuncture and other studies have localized the primary Na^+ handling abnormality to the distal nephron.^[150] Moreover, the reduction in GFR that is often present would further limit urinary Na^+ excretion and thus contribute to renal sodium retention.

The mechanism by which Na^+ handling is altered in the distal tubule is beginning to be understood. Considerable attention has focused on the role of ANP. Clinical^{[188] [189]} and experimental^{[190] [191]} studies have documented renal ANP resistance (i.e., blunted or absent natriuretic responses to

Figure 42-10 The "overflow" mechanism of edema formation. The abnormal renal Na^+ retention is viewed as the primary event that through the increased plasma volume leads to alteration of the Starling forces at the local tissue level. (From Perico N, Remuzzi G: *Edema of the nephrotic syndrome: The role of the atrial peptide system. Am J Kidney Dis* 22:355, 1993.)

ANP) in nephrotic syndrome. ANP resistance is confined to the ipsilateral kidney in unilateral glomerulonephritis,^[191] thus suggesting a role for this hormone in primary renal Na^+ retention. Although the mechanisms of ANP resistance are still being unraveled, some evidence relates this abnormality to heightened efferent sympathetic nervous activity.^[192] At the level of the tubule cell, evidence suggests that the problem is accelerated breakdown of normally produced cyclic guanosine monophosphate.^{[193] [194] [195]}

Recently, insight has been gained into the molecular mechanisms of renal sodium avidity. The hydrolytic and transport activities of sodium-potassium-adenosinetriphosphatase (Na^+ , K^+ -ATPase) are increased in the cortical collecting duct in nephrotic rats.^[196] The

proportional increases in $\text{Na}^+ , \text{K}^+ \text{-ATPase}$ activity, cell surface expression, and total cellular content are associated with increased amounts of α - and β -subunit mRNA.^[196] In principal cells from nephrotic rats, ENaC activity is increased in the absence of transcriptional induction of the mRNA encoding any of the ENaC subunits.^[197] Therefore, Na^+ retention in the cortical collecting duct appears to be due, at least in part, to coordinated overactivity of the $\text{Na}^+ , \text{K}^+ \text{-ATPase}$ and ENaC sodium transporters.^[196] Finally, a role for the proximal tubule has been invoked with the observation that Na^+ retention may also be associated with a shift of the cortical Na^+ / H^+ exchanger NHE3 from an inactive to an active pool.^[198]

Though less well studied, the mechanisms underlying abnormalities in water handling in experimental nephrotic syndrome have begun to be determined by recent molecular studies. These studies have noted reduced renal medullary water channel expression,^[199] impaired aquaporin and urea transporter expression,^[200] and decreased abundance of thick ascending limb Na^+ transporters.^[201]

Alterations in Renal Function

The Starling equation would predict that hypoalbuminemia and thus lower plasma colloid oncotic pressure would reduce the forces opposing ultrafiltration, thereby increasing glomerular filtration. However, clinical^[202] and experimental^{[203] [204]} studies indicate that such is not the case and that values of GFR are in fact reduced in conditions of reduced plasma protein levels. To examine the influence of plasma protein concentration on the determinants of glomerular filtration, Baylis and co-workers^[60] acutely changed the plasma protein concentration in normal rats. When the protein concentration was reduced, observed values for SNGFR were lower than predicted values; this failure of SNGFR to rise resulted from a concomitant reduction in K_f , the glomerular capillary ultrafiltration coefficient.^[60]

Reduced values of SNGFR, primarily caused by a reduction in K_f , have subsequently been observed in some,^[204] but not all^[205] experimental models of nephrotic syndrome; these differences in SNGFR derive, in part, from the presence or absence of compensatory elevations in the glomerular capillary hydraulic pressure ΔP . Studies in a unique strain of rats with no circulating albumin, the Nagase analbuminemic rat, have yielded further insight into the role of serum albumin in the regulation of GFR. Plasma oncotic pressure is modestly reduced in this strain, but a comparable reduction in Π_{IF} yields a fairly normal transcapillary $\Delta\Pi$ and normal extracellular fluid volume. GFR and renal plasma flow

values are comparable to those in normal rats; the constancy of the GFR relates in part to the elevation in K_f values because glomerular capillary pressure is somewhat reduced.^[206] These observations suggest that serum albumin per se may not have a direct effect on K_f or that other factors may mitigate the effects of hypoalbuminemia on K_f in the chronic setting.

Innovative methods for estimating values of SNGFR and its determinants in humans have also suggested that a reduction in K_f commonly accompanies clinical glomerulonephritis as well. For example, this pattern has been observed in patients with progressive lupus nephritis,^[207] sickle cell anemia,^[208] minimal change disease,^[209] and membranous nephropathy.^[210]

It should be noted that calculations of plasma oncotic pressure are generally performed by using the equation of Landis and Pappenheimer, in which the albumin-globulin ratio slightly exceeds unity.^[11] In the presence of severe hypoalbuminemia, this equation tends to overestimate values for colloid osmotic pressure and, therefore, to underestimate values for the ultrafiltration pressure used to calculate K_f . Accordingly, Miller and Meyer^[211] derived the equations required for modifying the Landis-Pappenheimer relationship in this setting. Subsequently, studies have confirmed that in nephrotic patients, π should be directly determined by membrane osmometry rather than calculated from the protein concentration.^[212]

Alterations in Drug Pharmacokinetics

Renal failure induces changes in all aspects of drug handling, including changes in bioavailability, the volume of distribution, renal drug metabolism, and renal excretion of drug or its metabolites, or both.^[213] Principles and guidelines for modification of drug dosage in renal insufficiency are readily available^[214] ^[215] and are detailed in [Chapter 66](#). The nephrotic syndrome poses special problems in drug handling and enhanced potential for both drug resistance and drug toxicity.

Hypoalbuminemia limits sites available for protein binding, thus increasing the amount of circulating free drug and potentially increasing first-pass hepatic drug removal. In addition, binding of organic acids and bases is altered in hypoalbuminemic states; the effect on organic acids is the more prominent.^[216] In nephrotic patients, reduced protein binding results both from hypoalbuminemia and from a decrease in albumin's affinity for drugs. Accordingly, the unbound fraction of acidic drugs, including salicylate and phenytoin, may be markedly increased.^[215] The clinical consequences of altered protein binding may be difficult to predict: decreased binding allows for a higher concentration of free drug, but this effect may be counteracted by a larger volume of distribution or faster metabolism of the drug (or both). Furthermore, protein binding may enhance tubule drug secretion; the lesser protein binding in nephrotic syndrome may result in delayed renal excretion of some drugs.^[213] Thus, in nephrotic patients, phenytoin is less protein bound, but the available free drug is more rapidly metabolized in the liver, so plasma levels are not elevated and the dosage need not be adjusted. In contrast, other protein-bound drugs, including prednisone and benzodiazepines, achieve significantly higher drug levels in nephrotic patients, with an enhanced risk of toxicity.^[215] Edema and ascites may increase the apparent volume of distribution of drugs that are highly water soluble or protein bound, thereby resulting in inadequate plasma levels; this effect is particularly prominent with aminoglycoside antibiotics.^[213]

The actions of diuretics are substantially altered in renal insufficiency and nephrotic syndrome, thereby contributing to the observed resistance to these drugs in this state.^[217]^[218]^[219]^[220]^[221]^[222] The unbound fraction of furosemide increases markedly in severely hypoalbuminemic patients.^[223] Nephrotic patients with a normal GFR deliver normal amounts of loop diuretics into the urine, but drug delivery is decreased in the setting of renal insufficiency.^[224] When proteinuria is also present, a substantial amount of furosemide may bind to urinary proteins, thereby reducing the amount of active, unbound drug in urine.^[225]^[226] Tubule albumin blunts the inhibitory effects of furosemide on fractional loop Cl⁻ reabsorption,^[227] whereas agents that block albumin-furosemide binding in the proximal tubule, such as warfarin and sulfisoxazole, partially restore diuretic responsiveness in experimental animals.^[228] However, a careful study found that sulfisoxazole was ineffective in nephrotic patients.^[229] Nephrotic patients exhibit abnormal pharmacodynamic responses to furosemide in addition to the binding effects,^[224] so the renal response to the drug is diminished even when adequate amounts of unbound, active drug reach the site of action. Furthermore, animal studies indicate that furosemide is less potent in inhibiting Cl⁻ reabsorption in the loop in nephrotic rats.^[230] Thus, both the pharmacodynamics and pharmacokinetics of loop diuretics are altered in nephrotic syndrome. Single intravenous doses of 80 to 120 mg may be required to attain therapeutic levels of furosemide in urine, but doses above this range are unlikely to achieve any added therapeutic response.^[218]^[221]

That hypoalbuminemia and altered protein binding are important factors in the resistance to diuretics is further supported by studies in a strain of analbuminemic rats.^[231] When compared with normal rats, these rats exhibited resistance to furosemide, with more rapid plasma disappearance of the drug and a larger total plasma clearance and volume of distribution. Injection of furosemide bound to albumin resulted in natriuresis, with normalization of the plasma disappearance rate and increased urinary excretion of furosemide. Thus, binding to plasma albumin appeared to be necessary for efficient delivery of drug into urine. These investigators then examined hypoalbuminemic patients with furosemide resistance and found that injecting furosemide as an admixture with equimolar albumin produced a diuresis whereas giving either alone was without effect. Whether natriuresis occurred in these patients was not specifically mentioned. Since that time, a preliminary report confirmed a natriuretic response to salt-free albumin mixed with bumetanide,^[232] but more recent studies in hypoalbuminemic cirrhotic patients found no benefit to combining albumin with furosemide.^[233]^[234] Because administration of large amounts of albumin alone is both ineffective and expensive, this therapeutic combination will require clear validation before its routine use can be recommended.

Therapy for glomerular disease or nephrotic syndrome may also be associated with drug interactions.^[215] For example, corticosteroids may inhibit hepatic microsomal enzymes,

thereby altering the metabolism of other drugs. Cyclophosphamide is not associated with significant drug interactions, but clinically important drug

interactions may be seen with other immunosuppressive drugs, including cyclosporine and azathioprine, as well as with diuretics and antihypertensive agents.^[215]

Alterations in Platelet Function

Hypoalbuminemia may contribute to abnormal platelet function in nephrotic patients because conversion of arachidonic acid to metabolites that aggregate platelets is regulated by albumin.^[235] In the presence of hypoalbuminemia, arachidonic acid may be metabolized to platelet-aggregating substances such as endoperoxides and thromboxane A₂.^[236] In support of this notion, the degree of platelet dysfunction tends to correlate with the severity of hypoalbuminemia and proteinuria.^[237] Platelets from nephrotic patients are refractory to adenylate cyclase stimulation by prostaglandin E₁, further enhancing the tendency toward increased platelet aggregation.^[238] However, a firm correlation between the plasma albumin concentration and platelet aggregability is not well established clinically.^{[239] [240]}

HYPERLIPIDEMIA

Hyperlipidemia is a frequent complication of nephrotic syndrome. Marked dysregulation of lipid metabolism occurs, with both quantitative and qualitative abnormalities in plasma lipids and lipoproteins. The precise pathogenesis,

Figure 42-11 Normal pathways of lipoprotein metabolism and potential derangements occurring in the nephrotic syndrome. (From Kaysen GA: *Hyperlipidemia in the nephrotic syndrome. Am J Kidney Dis* 12:548, 1988.)

clinical consequences, and optimal treatment of nephrotic hyperlipidemia are still being investigated. However, recent studies have identified a number of new mechanisms responsible for derangements of lipid metabolism in nephrotic syndrome. There is also increasing evidence of a broadening range of therapeutic options for these disorders. Although hyperlipidemia is associated with chronic renal diseases of every etiology,^{[241] [242]} it is most striking in nephrotic syndrome, where such changes occur even when the GFR remains normal.

Lipid Abnormalities in Nephrotic Syndrome

The nephrotic syndrome is characterized by abnormalities in virtually every aspect of lipid and lipoprotein metabolism, as depicted in [Figure 42-11](#).^{[243] [244] [245] [246]} Increased levels of the apolipoprotein B (apo B)-containing lipoproteins, very low density (VLDL), intermediate-density (IDL), and low-density (LDL) lipoproteins result in hypercholesterolemia, sometimes with hypertriglyceridemia. Cholesterol and phospholipid levels rise early in the course of the disease, whereas triglyceride (TG) elevations are more commonly found with more severe disease. Total HDL levels are usually normal, but in severely proteinuric patients, HDL may be lost in the urine, with

resultant reduced levels.^{[243] [244] [245] [246] [247] [248] [249]} Subtype analysis demonstrates an abnormal distribution with significant reductions in the protective subtype HDL2.^{[245] [248] [249]} Plasma concentrations of lipoprotein (a) [Lp(a)] are also elevated in nephrotic syndrome.^{[245] [246] [250] [251] [252] [253]} In addition, nephrotic patients show qualitative abnormalities in lipoprotein composition. The cholesterol-TG ratio is

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elevated in all classes of lipoproteins, which also tend to be enriched with cholesterol ester.^[245] The highly atherogenic small LDL-III fraction is elevated as well.^[254] The apolipoprotein content is also abnormal, with reduced apo C and E despite elevations in apo B, C-II, and E and an increased ratio of apo C-III to apo C-II.^{[247] [255] [256]} Taken together, these abnormalities result in an increased atherogenic profile.^{[247] [256] [257] [258]}

Pathogenesis of Nephrotic Hyperlipidemia

Nephrotic hyperlipidemia results from both overproduction and impaired catabolism or composition of serum lipids and lipoproteins. One of the major issues under investigation is whether the lipid abnormalities in nephrotic syndrome arise as a consequence of hypoalbuminemia or proteinuria. Early clinical studies demonstrated links between hypoalbuminemia per se and dysregulation of lipid metabolism in nephrotic syndrome. In general, the severity of hyperlipidemia tends to correlate with the severity of hypoalbuminemia. In addition, remission of nephrotic syndrome is usually associated with a decrease in serum cholesterol as the albumin level rises, whereas albumin infusion acutely raises serum albumin and lowers serum cholesterol levels.^{[98] [248] [259] [260]} Because hepatic synthetic rates of albumin and lipoproteins react to similar stimuli and follow the same synthetic pathways, it has been hypothesized that increased lipoprotein synthesis was simply a side effect of increased albumin synthesis. However, although albumin synthesis is increased, no clear correlation has been found between hyperlipidemia and the rate of albumin synthesis in nephrotic patients. Kaysen and colleagues^[261] showed that serum cholesterol levels in nephrotic patients were dependent only on the renal clearance of albumin and were totally independent of albumin synthetic rates but that serum TG levels showed some dependence on albumin synthesis. Similarly, serum lipid levels in nephrotic rats correlated with proteinuria and not with albumin synthetic rates.^[262] An alternative stimulus may be the reduction in plasma oncotic pressure. Infusion of either albumin or dextran into nephrotic patients and animals reduces serum lipid levels, thus suggesting that low plasma oncotic pressure may stimulate hepatic lipoprotein synthesis.^{[260] [263] [264]}

Intensive research in this area has identified numerous pathogenetic mechanisms responsible for these complex alterations in lipid metabolism. It is now apparent that reductions in plasma albumin levels or oncotic pressure, as well as the direct consequences of proteinuria, contribute to lipid alterations in nephrotic syndrome. As discussed later, these major factors operate on various levels of the lipid metabolic

pathways. Metabolism of lipoproteins is closely linked. For purposes of this review, defects in the metabolism of individual fractions will be discussed separately. However, the reader should be aware that one mechanism may alter the levels and composition of multiple lipoproteins.

Alterations in Low-Density Lipoprotein Metabolism

Increased synthesis seems to be the principal mechanism responsible for the higher levels of LDL in nephrotic syndrome. It has been proposed that some nephrotic patients have increased absolute LDL apo B-100 synthetic rates, greater than VLDL apo B-100 and independent of VLDL synthesis, thus suggesting an alternative secretory pathway for LDL that bypasses VLDL delipidation. Previous studies of LDL metabolism have produced conflicting results, many of which could result from methodologic differences.^[265] ^[266] Recent studies have avoided some of the problems involving measurement of apo B-100 synthesis by using endogenous labeling of lipoproteins with valine labeled with stable isotopes. Importantly, increased LDL apo B synthesis did not correlate with the synthetic rate of albumin.^[265]

Plasma levels and the activity of cholesterol ester transfer protein (CETP) are enhanced in nephrotic syndrome.^[267] This protein mediates the transfer of esterified cholesterol from HDL to VLDL remnants to yield LDL. In addition to the defects discussed earlier, acquired defects in LDL clearance have also been described. Some studies have demonstrated reduced receptor-mediated LDL clearance with associated increases in LDL catabolism via alternative pathways.^[268] ^[269] Increases in hepatic cholesterol concentrations could contribute to hyperlipidemia both by increasing VLDL production and by down-regulating expression of LDL receptors.^[245] Others have suggested that LDL kinetics and pathogenetic mechanisms differ markedly in patients with hypercholesterolemia alone versus those with concurrent hypertriglyceridemia, with overproduction of LDL found only in patients with combined hyperlipidemia.^[270]

Alterations in Very Low Density Lipoprotein Metabolism

The increased VLDL levels in nephrotic syndrome occur predominantly as a result of impaired VLDL clearance. Early studies demonstrated defective chylomicron clearance in nephrotic rats.^[262] This phenomenon correlated with proteinuria rather than hypoalbuminemia because the serum half-life of chylomicrons remained normal in analbuminemic rats whereas it was markedly impaired in hypoalbuminemic nephrotic animals. In addition, plasma TG levels are higher in nephrotic than in analbuminemic rats despite similar increases in hepatic TG production.^[271] Defective VLDL clearance has also been documented in nephrotic patients.^[265]

As a major determinant of chylomicron and VLDL clearance, the functional integrity of lipoprotein lipase (LPL) has been a logical focus for study in this area. Reduced LPL activity in nephrotic patients was proposed by Garber and associates.^[272] Earlier reports suggested that decreased LPL activity may relate to the increased levels of circulating free fatty acids that result from hypoalbuminemia and the lowered protein-binding

capacity of plasma. The increased free fatty acid level contributes by providing the lipid substrate for increased hepatic lipoprotein synthesis and by leading to decreased activity of LPL.^{[273] [274]}

LPL is attached to the endothelium by ionic bonding to a negatively charged matrix of glycosaminoglycans such as heparan sulfate.^{[3] [273]} This endothelium-bound LPL is an active, metabolically important pool, and earlier studies demonstrated that it is reduced in nephrotic rats^{[262] [275]} and patients.^[276] Urinary excretion is markedly increased in nephrotic patients,^[274] and circulating levels of heparan sulfate are reduced in nephrotic plasma and contribute to the decrease in LPL activity.^[3] In support of this concept, studies

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in nephrotic rats show that the markedly delayed plasma disappearance of radiolabeled chylomicrons may be completely normalized by injection of minute amounts of purified urinary heparan sulfate.^{[3] [277]} The heparan sulfate deficiency may also result from deficient hepatic synthesis of glycosaminoglycans. Nephrotic syndrome is characterized by excessive urinary losses of orosomucoid, a plasma glycoprotein synthesized by the liver. Urinary losses may lead to an increase in hepatic synthesis with a resultant excessive drain of key sugar intermediates from liver parenchymal cells, thus limiting the substrates available for heparan sulfate synthesis.^[3] Because the endothelial pool of LPL in Nagase analbuminuric rats is reduced to the same extent as in nephrotic rats but TG levels are much higher in the latter model, it has been hypothesized that in addition to defects in endothelial LPL, other important determinants of VLDL levels are present in nephrotic syndrome.

Indeed, more recent studies have revealed abnormalities in other determinants of VLDL clearance. In two different models of nephrotic syndrome, Liang and Vaziri^{[278] [279]} demonstrated that elevated serum TG levels are in part attributable to reduced VLDL receptor and LPL expression. Reductions in VLDL receptor protein and mRNA were inversely related to plasma VLDL and TG concentrations. The same group implicated secondary hyperparathyroidism in the reduced LPL and hepatic lipase activity of proteinuric rats with progressive renal failure and suggested that because of depletion of hepatic LPL in nephrotic rats, there is no liver compensation for the LPL defect.^[280] Furthermore, defective receptor-mediated clearance and a metabolic defect in recognition and removal by the liver may underlie the elevated remnant particles in nephrotic syndrome.^{[3] [281]} The signal for recognition of VLDL remnants by a liver "receptor site" may be LPL, an associated cofactor such as heparan sulfate, or certain apolipoproteins, and it has been hypothesized that the observed deficiencies in LPL or heparan sulfate (or both) may be responsible for failure of recognition and, hence, defective removal of these particles.^[3]

VLDL isolated from nephrotic rats hydrolyzes at a different rate in *in vitro* systems than it does in control animals.^[282] Shearer and colleagues^[283] perfused hearts from normal,

analbuminemic, and nephrotic rats with chylomicrons and found identical clearance of these particles in analbuminemic and nephrotic rats. These observations clearly suggest that altered structure or composition of TG-rich lipoproteins must play a role in altered VLDL clearance. In both studies, the defects in lipolysis in nephrotic rats were corrected by HDL, thus suggesting that a component within HDL played a role in the pathophysiology of these alterations. To facilitate VLDL receptor-mediated and LPL-mediated clearance, HDL supplies VLDL with most of the apo E and apo C. Alterations in these molecules in nephrotic syndrome have been described; apo E is reduced in the HDL of nephrotic rats and in the VLDL of nephrotic patients.^[256] Apo C has been found to be markedly reduced per unit of VLDL in nephrotic patients^{[255] [256]} despite normal or even increased plasma levels. Reductions in VLDL apo C and apo E correlate with particle size.^[256] Thus, in addition to reduced LPL activity, VLDL clearance in nephrotic syndrome is delayed because of altered composition.

VLDL synthesis has been also evaluated. Earlier reports suggested increased VLDL synthesis related at least in part to reductions in plasma albumin or oncotic pressure.^{[261] [262] [271]} Furthermore, apo B synthesis may be increased in some nephrotic patients.^{[265] [284]} Experimental support for increased VLDL synthesis comes from the observation that levels of apo B mRNA are reduced in cultured hepatocytes when albumin or dextran is added to the medium and increased in the presence of hypo-oncotic medium.^{[111] [285] [286]} However, more recent data indicate normal or even decreased VLDL secretion and no correlation with albumin synthesis in experimental and clinical nephrotic syndrome.^{[265] [283]}

Alterations in High-Density Lipoprotein

Nephrotic syndrome is associated with specific abnormalities in enzymatic functions required for effective function of HDL. Diminished activity of the enzyme lecithin-cholesterol acyltransferase (LCAT) appears to contribute to the lipoprotein abnormalities in nephrotic syndrome.^{[287] [288] [289]} LCAT is involved in catalyzing the esterification of cholesterol and its incorporation into HDL particles, as well as the conversion of HDL3 to HDL2. Low LCAT levels would impair this HDL maturation, in turn reducing the transfer of apo C-II to VLDL and thus inhibiting the catabolism of TG-rich lipoproteins.^[245] Nephrotic patients have a distribution in HDL isoforms that corresponds to the LCAT defect—the higher-molecular-weight HDL2 is reduced and replaced by an increase in the lower-molecular-weight HDL3. Recent observations have demonstrated that the LCAT deficiency in nephrotic rats is due to urinary losses.^[289] In contrast to nephrotic rats, Nagase analbuminemic rats do not demonstrate substantial LCAT alterations. However, earlier studies suggested that hypoalbuminemia could also play a role by increasing levels of free (unbound) lysolecithin, an inhibitor of LCAT.^{[3] [290]}

Increased hepatic production and elevated plasma CETP levels may contribute to HDL abnormalities in nephrotic patients.^[267] As a mediator of transfer of esterified cholesterol from HDL to VLDL, elevated levels might contribute to cholesterol enrichment of TG-rich lipoproteins, as well as the observed reductions in HDL2.^{[267] [288] [291]} Braschi and co-workers^[267] demonstrated that the enhanced CETP activity in nephrotic patients could be

attributed to marked increases in the proportion of lipoprotein-bound nonesterified fatty acids that are responsible for increases in the negative charge of nephrotic lipoproteins.

Elevated HDL in nephrotic rats is associated with apo A-I enrichment of HDL particles.^{[292] [293]} This abnormality has been linked to hypoalbuminemia and reduced oncotic pressure, and the accumulation of apo A-I—rich HDL is due to increased hepatic synthesis and reduced catabolism of HDL and apo A-I.^{[292] [293]} Importantly, the relevance of these observations for human studies is unknown. Unlike experimental models, fractional apo A-I in nephrotic patients is increased because of the increase in CETP that is absent in rats. CETP mediates conversion of the larger HDL2 to the smaller HDL3, which has less affinity for apo A-I, and thus indirectly facilitates clearance of apo A-I.^[294] Finally, the altered plasma HDL levels and composition in nephrotic rats are at least partly attributable to reduced protein expression

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of SR-B1.^[295] This molecule has been identified as an HDL receptor responsible for the clearance of these particles.

Lipoprotein (a)

Persuasive evidence indicates that Lp(a) is increased in nephrotic patients.^{[245] [246] [251] [252] [253]} In view of the atherogenic potential of Lp(a), these findings are important. The principal mechanism leading to elevations in Lp(a) in nephrotic patients seems to be increased synthesis alone.^{[251] [253]} Lp(a) is related to apo B synthesis in nephrotic humans.^{[252] [253]} As demonstrated by Noto and colleagues,^[252] Lp(a) levels in nephrotic children inversely correlate with apo(a) isoform size and plasma albumin levels, but not with the proteinuria-creatinine clearance ratio.

Cholesterol Synthesis

Cholesterol content is increased in most of the nephrotic lipoproteins. These abnormalities could be explained by some of the abnormalities in lipoprotein function and composition discussed earlier. In addition, some evidence indicates enhanced hepatic cholesterol synthesis in experimental nephrotic syndrome. One factor that might contribute to increased cholesterol synthesis is greater availability of the cholesterol precursor mevalonic acid. Circulating mevalonate is metabolized in the kidney, and both renal excretion and renal metabolism of this substance are impaired in the nephrotic state.^[296] Several studies have demonstrated enzymatic defects in the liver of nephrotic rats that can collectively enhance hepatic cholesterol synthesis. These studies have shown increased hepatic activity of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, in nephrotic rats.^{[245] [296] [297]} In contrast, hepatic expression of cholesterol 7 α -hydroxylase, the rate-limiting enzyme responsible for conversion of cholesterol to bile acids, is reduced in nephrotic rats.^[298] Most recently, marked up-regulation of hepatic acetyl coenzyme A: cholesterol acyltransferase (ACAT)

has been described in nephrotic rats.^[289] This multifunctional enzyme is involved in the catalysis of intracellular cholesterol esterification and is responsible for lowering intracellular free cholesterol. By lowering hepatic free cholesterol, ACAT up-regulation may be responsible for the aforementioned defects in HMG-CoA reductase and 7 α -hydroxylase activity and the enhanced cholesterol synthesis. Furthermore, enhanced ACAT activity leads to intracellular accumulation of cholesterol ester. Increases in hepatic cholesterol concentrations could contribute to hyperlipidemia both by increasing VLDL production and by down-regulating the expression of LDL receptors.^[245] In the vascular system, this phenomenon leads to foam cell formation and atherosclerosis.^{[299] [300]} It should be noted that increases in expression or activity of hepatic HMG-CoA reductase in animals with nephrotic syndrome are not uniform findings. Thabet and co-workers^[301] did not find persistently increased mRNA liver expression of this enzyme, thus suggesting that an increase in cholesterol biosynthetic capacity is not necessary for maintenance of nephrotic hypercholesterolemia.

Results of studies in humans are contradictory. Turnover studies using radiolabeled glycerol and mevalonate have suggested increases in cholesterol synthesis.^[245] In contrast, the serum lathosterol-to-cholesterol ratio, an index of cholesterol synthesis, is not elevated and does not change in response to antiproteinuric treatment.^[302] Whether increased cholesterol synthesis actually occurs in human nephrotic syndrome therefore remains unclear.

Clinical Consequences of Nephrotic Hyperlipidemia

Many of the lipid abnormalities in nephrotic syndrome are significant risk factors for atherosclerotic cardiovascular (CV) disease in the general population, including increases in total cholesterol, LDL- and VLDL-cholesterol, apo B, and Lp(a) and reductions in HDL2 cholesterol. Furthermore, additional risk factors, such as hypertension, endothelial dysfunction, and hypercoagulability, may also contribute to the risk of atherosclerotic CV disease. A small study found elevated plasma homocysteine levels in nephrotic patients as well.^[303] Nonetheless, evidence that CV risk is indeed increased in these patients remains controversial, and prospective long-term data are not available. Studies that have tried to define CV risk in nephrotic patients have been flawed by inclusion of patients with minimal change disease, which typically remits; diabetes, which is inherently atherogenic; or failure to control for the presence of other risk factors. Early studies, which included relatively young patients, contained small numbers, and were retrospective in design, have not uniformly found an increased risk of CV events.^{[304] [305] [306]} However, in a retrospective analysis of 142 nephrotic patients without diabetes, Ordonez and colleagues^[307] found that after correction for hypertension and smoking, the relative risk of myocardial infarction was increased 5.5-fold and that of coronary death was increased 2.8-fold in comparison to non-nephrotic controls. In addition, Falaschi and co-workers^[308] evaluated the carotid intima-media wall thickness (IMT) in young patients with lupus as a marker of early atherosclerosis and CV risk. Patients with nephrotic-range proteinuria had a significantly higher IMT than did those without. The IMT did not

correlate with the lupus activity score or other possible risk factors except for proteinuria, thus suggesting a higher risk of early atherosclerosis even in this young age group.^[308]

Recent studies have focused on alterations in endothelial function associated with nephrotic syndrome. These complex changes with multifactorial etiology may be a common denominator of the clinical consequences of nephrotic syndrome, such as atherosclerosis, hypertension, and hypercoagulability. Nephrotic patients may exhibit impaired endothelium-dependent relaxation^[309] ^[310] and decreased total plasma antioxidant potential.^[311] Hyperlipidemia itself is also a risk factor for impaired endothelial function. Altered lysophosphatidylcholine metabolism, linked to both hyperlipidemia and hypoalbuminemia, is another factor responsible for the endothelial dysfunction in nephrotic syndrome.^[312]

Hyperlipidemia probably contributes to other adverse consequences of nephrotic syndrome. The increased platelet aggregation tends to correlate with the magnitude of hyperlipidemia.^[236] Hyperlipidemia may also contribute to the increased susceptibility of nephrotic patients to infection inasmuch as serum from nephrotic patients inhibits lymphocyte proliferation in response to specific and nonspecific

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antigen stimulation.^[313] In addition to increasing the risk for CV disease, Lp(a), which may act to inhibit plasminogen, could contribute to hypercoagulability. Finally, the role of hyperlipidemia as a risk factor for progression of glomerular injury is discussed in detail in [Chapter 43](#) .

Therapy for Nephrotic Hyperlipidemia

In view of the magnitude of the CV risk in this population, further studies are needed to establish the need for aggressive hypolipidemic therapy.^[257] In general, attempts to modify the lipoprotein profile may be worthwhile in patients with unremitting nephrotic syndrome, particularly if other CV risk factors are present. The principles of therapy are similar to those in other populations and include alterations in diet, the use of pharmacologic agents, and attention to other CV risk factors. Although few studies have systematically looked at the impact of standard dietary therapy in proteinuric patients, a moderate reduction in dietary cholesterol intake appears to be relatively ineffective.^[314] Studies of vegetarian soy diets that are low in protein and rich in monounsaturated and polyunsaturated fatty acids have demonstrated improvements in serum cholesterol, LDL, and apo B in patients with proteinuria.^[315] Supplementation of this diet with fish oil was not of additional benefit,^[316] although it may provide some beneficial effect on TG levels.^[245] ^[315]

More promising are pharmacologic approaches, particularly the statins. Fibric acid derivatives have a more prominent effect on TG metabolism than on cholesterol. Early studies with clofibrate found a frequent complication of muscle toxicity.^[317] In one study

of 11 patients treated with gemfibrozil, TG levels fell and HDL levels rose, with little change in total cholesterol or LDL-cholesterol levels.^[318] Controlled prospective studies have indicated that colestipol and probucol may also have modest hypolipidemic effects.^{[319] [320]}

The preferred agents in nephrotic patients are HMG-CoA reductase inhibitors, which induce the greatest and most consistent hypolipidemic effect.^{[246] [320]} Lovastatin, simvastatin, and pravastatin are all beneficial and well tolerated and result in reductions in total cholesterol, LDL, apo B-100, and TG and increases in HDL.^{[321] [322] [323] [324] [325] [326] [327] [328]} Lp(a) levels may also be reduced by statins.^{[329] [330]} However, the literature regarding Lp(a) is inconsistent. In the largest reported study, Olbricht and co-authors^[328] conducted a prospective, randomized, placebo-controlled trial of 102 patients with glomerulonephritis and at least 3 g of proteinuria per day. With simvastatin, mean changes from baseline in total cholesterol, LDL-cholesterol, HDL-cholesterol, and serum TG were -39%, -47%, +1%, and -30%; serum Lp(a) was not affected. The final outcomes of the study have not yet been reported. Another recent study demonstrated a possible benefit of combinations of statins with fibrates.^[331] Other than lipid lowering, the beneficial effects of statins may include a reduction in platelet aggregation and procoagulant factors, inhibition of mesangial cell proliferation and matrix accumulation, and anti-inflammatory effects.^[246]

In addition to standard hypolipidemic therapies, interventions that reduce proteinuria may also indirectly improve serum lipid profiles. Several studies of ACE inhibitor therapy have demonstrated improvement in lipid profiles, including reductions in Lp(a).^{[332] [333] [334] [335]} Similar changes occur after the administration of losartan, an AII receptor antagonist.^[336] Finally, several reports have indicated beneficial effects of lipoprotein apheresis on severely hyperlipidemic nephrotic patients,^{[337] [338] [339]} although evidence of long-term outcomes from this intervention are currently lacking.^[340]

HYPERTENSION

Hypertension frequently accompanies glomerular diseases with a nephritic pattern and may accompany nephrotic diseases as well. Although exceptions exist, hypertension in the absence of renal insufficiency is more likely to be present in primary glomerular diseases than in diseases of tubulointerstitial origin. The relationship between hypertension and glomerular disease has been the subject of several reviews^{[341] [342] [343]} and is discussed in detail in [Chapter 47](#) .

Multiple factors are likely to play a role in the pathogenesis of hypertension associated with glomerular disease.^[344] In patients with severe renal functional impairment and with an acute nephritic syndrome and extracellular volume expansion, hypertension is generally volume dependent and is responsive to interventions that ameliorate the volume overload.^{[151] [345]} In addition, elevated peripheral vascular resistance may contribute to hypertension, even in the presence of volume expansion. Although absolute values of plasma renin and AII in such patients may be normal, they are also inappropriately high for the degree of PV expansion, thus suggesting resetting of the Na⁺ -volume-renin feedback mechanism.^{[344] [345]} In nephrotic syndrome, hypertensive patients also appear to

fall in the group with PV expansion,^{[166] [167]} with blood pressures falling after remission or diuretic therapy.^[346] Though not well studied, urinary loss of an antihypertensive substance is a possibility. For example, nitric oxide circulates bound to albumin.^[347]

HEMATOLOGIC ABNORMALITIES (See also [Chapter 49](#))

Hypercoagulable State and Renal Vein Thrombosis

The nephrotic syndrome is frequently complicated by an enhanced tendency for intravascular coagulation, with a consequent risk of thromboembolic complications. The most common manifestation is the development of renal vein thrombosis, which is most frequently associated with membranous glomerulonephritis. Prospective studies of the incidence of renal vein thrombosis in patients with membranous nephropathy indicated an average incidence of about 35%, with individual studies finding a range of 5% to 62%.^{[236] [348] [349]} The incidence is much lower in other forms of glomerulonephritis, for unknown reasons.

Thrombosis is not limited to the renal venous circulation, although this site predominates. The incidence of thrombotic complications at other sites ranges from 8% to 44%, with an average of about 20%.^{[348] [349] [350]} Of such complications, pulmonary embolism is the most frequent and serious. In a study of 204 children and 116 adults with nephrotic syndrome, children exhibited a lower incidence of events than adults did.^[351] However, the complications tended to be more severe in children, almost half of whom exhibited arterial

Figure 42-12 Schematic representation of pathogenetic factors leading to hypercoagulability, thromboembolic phenomena, and renal vein thrombosis in nephrotic syndrome. (From *Llach F: Hypercoagulability, renal vein thrombosis, and other thrombotic complications of nephrotic syndrome. Kidney Int 28:429, 1985.*)

thrombosis.^[351] As mentioned earlier, the relative risk of coronary thrombotic events is increased in these patients,^[307] and hypercoagulability could well contribute to this finding.

Pathogenesis of Hypercoagulability

The numerous abnormalities in the coagulation and hemostasis systems that accompany nephrotic syndrome have been extensively reviewed^{[94] [95] [236] [348] [352]} and will be briefly summarized here. These abnormalities include alterations in the levels and activity of factors in the intrinsic and extrinsic coagulation cascades, levels of antithrombotic and

fibrinolytic components of plasma, platelet counts and platelet function, blood viscosity, and other factors. A pathogenetic mechanism for these abnormalities is depicted in [Figure 42-12](#),^[236] and reported abnormalities are summarized in [Table 42-1](#). As reviewed by Llach,^[236] abnormalities of coagulation in the nephrotic syndrome may relate to each of the five major functional classes of coagulation components: (1) zymogens (factors II, V, VII, IX, X, XI, and XII), which are activated to enzymes, and cofactors (factors V and VIII), which accelerate the conversion of zymogens; (2) fibrinogen; (3) the fibrinolytic system; (4) clotting inhibitors; and (5) components of platelet reaction and thrombogenesis.

TABLE 42-1 -- Coagulation Abnormalities in Nephrotic Syndrome
Alterations in zymogens and cofactors
Deficiency in factors IX, XI, XII
Increased levels of factor II and combined factors VII and X
Increased levels of factors V and VIII
Increased plasma fibrinogen levels
Alterations in the fibrinolytic system
Deficiency of plasma plasminogen
Low antiplasmin activity (α_1 -antitrypsin)
Increased antiplasmin activity (α_2 -macroglobulin fraction)
Increased α_1 -antiplasmin
Alterations in coagulation inhibitors
Deficiency of antithrombin III
Deficiency of protein S
Deficiency of protein C (possible)
Alterations in platelet function
Enhanced platelet aggregability
Increased levels of β -thromboglobulin
<i>Data modified from Llach F: Hypercoagulability, renal vein thrombosis, and other thrombotic complications of nephrotic syndrome. Kidney Int 28:429, 1985.</i>

Alterations in Zymogens and Cofactors

Most studies have noted deficiencies in levels of factors IX, XI, and XII,^{[352] [353] [354] [355] [356] [357]} which are likely to relate to urinary loss of these low-molecular-weight proteins. Deficient factor XII levels are particularly important because this factor regulates

coagulation activity, as well as the fibrinolytic and kinin-kallikrein pathways.^[358] Increases in the level of factor II and combined factors VII and X have also been noted.^[359] These zymogen abnormalities usually normalize with clinical remission of nephrotic syndrome.^[353] The nephrotic syndrome is also characterized by increased levels of the cofactors V and VIII, which may correlate inversely with the level of serum albumin.^[359]^[360]^[361] The serum elevations appear to result from increased hepatic synthesis, perhaps in response to the decreased oncotic pressure or hypoalbuminemia (or both). These abnormalities in zymogens and cofactors have not been clearly associated with thrombotic complications.^[236]

Alterations in Fibrinogen Levels and the Fibrinolytic System

The nephrotic syndrome is associated with elevated plasma fibrinogen levels,^[351]^[359]^[360]^[361] which most probably result from increased hepatic synthesis and normal catabolic rates.^[362] Fibrinogen levels correlate directly with urinary protein and serum cholesterol levels and inversely with serum albumin levels.^[359]^[360]^[361] Fibrinogen is an important determinant of plasma viscosity, and the increased levels may be of pathogenetic importance in the hypercoagulability of nephrotic syndrome. Indeed, by inducing fibrin deposition, hyperfibrinogenemia may be a major factor determining thrombotic risk.^[363]

The data on fibrinolytic abnormalities, which are associated with thrombosis in other conditions, are conflicting in nephrotic syndrome. Several studies have found deficiencies in plasma levels of plasminogen, with the decrease correlating with the magnitude of hypoalbuminemia and proteinuria.^[364]^[365]^[366] Other reported abnormalities include low antiplasmin activity (α_1 -antitrypsin)^[360] and increased antiplasmin activity (α_2 -macroglobulin fraction, which is the primary plasmin inhibitor and may be the most reliable marker of renal vein thrombosis).^[367]

Alterations in Coagulation Inhibitors

Nephrotic patients exhibit increased urinary losses and decreased plasma levels of the protease inhibitor antithrombin

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III (ATIII), the most important inhibitor of coagulation and thrombin.^[351]^[368] Deficient serum levels of ATIII are sometimes,^[367] though not always^[369] correlated with thromboembolic phenomena in nephrotic patients. ATIII deficiency, a defect that is reversible with steroid therapy,^[370] occurs commonly, but not universally in nephrotic syndrome.^[236]

Abnormalities in other coagulation inhibitors, including protein C and protein S, may also occur in nephrotic syndrome; congenital deficiencies of each of these proteins are associated with recurrent venous thrombosis.^[371]^[372] Both these proteins are found in the urine of nephrotic patients.^[373]^[374] Levels of total protein S and protein C antigens are

elevated, but the activity of protein S is reduced because of a significant reduction in free (active) protein S levels, a consequence of elevated urinary losses.^[374] Protein C anticoagulant activity is elevated, although a marked reduction in specific activity has been noted. Nephrotic patients may exhibit elevations in serum thrombin activatable fibrinolysis inhibitor (TAFI), as well as a deficiency in protein Z, two additional factors that may predispose to thrombosis.^[375] A reduction in tissue factor pathway inhibitor (TFPI) has been postulated, but one study found that proteinuria was in fact associated with increased TFPI levels, so the thrombotic tendencies cannot be ascribed to TFPI deficiency.^[376]

Alterations in Platelet Function

Platelet counts in nephrotic patients tend to be normal or elevated.^{[359] [360]} Platelet aggregability may be increased^{[240] [369] [377]}; the potential contributions of hyperlipidemia and hypoalbuminemia to this abnormality are discussed earlier. Nephrotic patients may also exhibit elevations in β -thromboglobulin, a specific protein released by platelets on aggregation.^{[378] [379]}

In summary, numerous coagulation abnormalities are found in nephrotic syndrome. In addition to the factors described earlier, nephrotic syndrome may be characterized by increased blood viscosity^{[379] [380]} as a result of both hyperlipidemia and increased fibrinogen. Steroid therapy may also exacerbate hypercoagulability in nephrotic patients.^[381]

The specific role of each of these abnormalities in the pathogenesis of thromboembolic complications remains incompletely defined.^[236] An increased tendency toward thrombotic events has been correlated with increased α_2 -antiplasmin levels,^[367] and the presence of factor XII and prekallikrein in subepithelial deposits has been noted in patients with membranous glomerulonephritis.^[382] However, a prospective study of nephrotic adults monitored for an average of 21 months found significant increases in factor I, factor VIIIc, factor VIII:Ag, α_1 -antitrypsin, and α_2 -macroglobulin, as well as platelet hyperaggregability, in the group as a whole, but no correlation between these abnormalities and thromboembolic events. Low levels of ATIII and severe hypoalbuminemia were of no predictive value for thromboembolic events.^[369] Of five patients with three potential risk factors (severe hypoalbuminemia, low ATIII levels, and platelet hyperaggregability), none had thromboembolic complications during the course of the study. Thus, although the nephrotic syndrome features prominent hematologic abnormalities and a tendency toward thromboembolic complications, the relationship between these problems remains to be completely defined.

HORMONAL AND OTHER SYSTEMIC MANIFESTATIONS

Other systemic manifestations of glomerular disease, which are covered in detail elsewhere in this volume, include enhanced susceptibility to infection,^{[96] [98] [383]} possibly as a result of urinary loss of components of the alternative complement pathway, including factor B, and loss of IgG.^[383] IgG synthesis may also be impaired.^{[384] [385]} Deficiencies of

trace metals such as copper, zinc, and iron may occur.^{[386] [387] [388] [389]} Urinary losses of thyroxine-binding globulin, triiodothyronine, and thyroxine have been noted, although patients remain clinically euthyroid.^{[390] [391]} Urinary levels of corticosteroid-binding globulin^[392] and insulin-like growth factor type I^[393] are elevated, although the clinical consequences are unclear. Abnormalities in Ca²⁺ and vitamin D metabolism, such as hypocalcemia, hypocalciuria, and low serum levels of vitamin D, also characterize the nephrotic syndrome.^{[98] [394]} It is not clear that clinically significant hypovitaminosis D occurs in the majority of nephrotic patients,^[95] but a recent study found an increased incidence of isolated osteomalacia and bone resorption in association with defective mineralization in patients with sustained nephrotic syndrome.^[395] Urinary levels of erythropoietin are increased, and plasma levels fail to rise despite anemia^[396] ; erythropoietin deficiency can occur and cause anemia even in the setting of normal kidney function.^[397] Transferrin synthesis is increased, but not sufficiently to replace urinary losses.^[398] Finally, extrarenal protein loss in the presence of inadequate protein intake may be associated with negative nitrogen balance and protein malnutrition.^[99]