Properties of the Glomerular Barrier and Mechanisms of Proteinuria

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I. INTRODUCTION

The intricate properties of the glomerular barrier have fascinated researchers for decades (87). Today, many of the molecular components of the barrier have been revealed (234), but their functional interactions remain to be elucidated. The glomerular barrier is by far the most complex biological membrane, with properties that allow for high filtration rates of water, nonrestricted passage of small and middle-sized molecules, and almost total restriction of serum albumin and larger proteins. In humans, close to 180 liters of primary urine are produced each day at capillary pressures far exceeding those in other organs. Despite this tremendous work load, the glomerulus remains intact year after year. Indeed, several anticlogging mechanisms have been proposed, including protein consumption by the mesangial cells (87), charge repulsion in the glomerular basement membrane (GBM) (146), a tangential-flow filtration mechanism (260), and a gel permeation hypothesis (292, 346, 347).

This review focuses on the functional properties of the glomerular barrier and the mechanisms of proteinuria and nephrotic syndromes. Although recent discussions of proteinuria have tended to focus on the role of the podocyte (319), a more complete understanding of the underlying mechanisms requires careful consideration of the other glomerular components (323). To this end, we provide an overview of the role of glomerular endothelial cells and the glycocalyx surrounding them and discuss the GBM. For details on podocyte biology, readers are referred to Pavenstädt et al. (234). Also considered are interactions between the barrier and plasma proteins, such as orosomucoid and albumin, which further illustrate the multifaceted nature of the glomerular membrane. The principal components of the glomerular barrier are illustrated in Figure 1, together with the approximate glomerular filtration rate, plasma flow rate, and albumin concentrations in humans.

Next we review the various methods used to study glomerular permeability and discuss their virtues and limitations. Misconceptions about the properties of the macromolecular tracers used to analyze permeability have led to some confusion in the field. Therefore, we discuss the “ideal solute” and compare its properties with those of the solutes currently available for research. Subsequently, we address the renal clearance of various solutes in humans and intact animals and under different experimental conditions, discussing the results in terms of solute size, shape, and charge. At the end of that section, the recent controversy on glomerular charge selectivity is discussed and analyzed.

Finally, we review physical principles and various theoretical models that reflect our understanding of how the glomerular barrier carries out its remarkable functions. “Simple” and multilayer barriers are discussed,
along with the implications of their behaviors for interpreting in vivo and in vitro data.

II. COMPONENTS OF THE GLOMERULAR BARRIER

A. Podocytes

The epithelial cells of the glomerulus have attracted considerable interest in recent years. These specialized, highly differentiated cells line the outside of the glomerular capillaries and thus face the Bowman’s capsule and the primary urine. The cells have a large cell body and long, extending cytoplasmic foot processes, which are separated by a filtration slit that is ~25–60 nm wide (150, 269, 342) and covered by a diaphragm. The molecular components of the diaphragm have been extensively studied, and some proteins are vital for the maintenance of normal glomerular permselectivity: the restriction of the permeation of macromolecules across the glomerular barrier on the basis of molecular size, charge, and physical configuration. One such molecule is nephrin, which when mutated causes massive leakage of protein and severe consequences for patients with congenital nephrotic syndrome of the Finnish type (153, 154).

The podocyte is a major component of the glomerular barrier, but its contribution to the restriction of fluid and protein transport is unclear. The slit membrane has porelike structures (342) whose dimensions are postulated to be 40 × 140 Å (254, 319), corresponding to a slit half-width of 20 Å. With a Stokes-Einstein radius (the most common measure of molecular size; see sect. viA) of 36 Å, albumin cannot reach the urinary space across such slits, except through “large pores” or “shunt pathways.” Moreover, the slit membrane dimensions described above also are too small to explain the sieving of other solutes. The sieving coefficient (Θ) of a solute is a measure of its transport rate in relation to that of water. Myoglobin with a Stokes-Einstein radius of 18 Å has a glomerular sieving coefficient close to 0.8 (348), but a slit half-width of 20 Å implies total restriction (116), i.e., Θ close to zero. It is therefore most likely that the slit dimensions were underestimated in Reference 254. In addition, several studies in both mice and humans show that proteinuria can occur without effacement of podocyte foot processes (296, 330). Indeed, proteinuria may occur regardless of which layer of the glomerular wall is damaged (116, 144).

B. Basement Membrane

The basement membrane is composed of a fibrous network consisting of type IV collagen (collagen α3, α4, and α5 chains) (193, 271), laminin (mainly laminin 11; α5βγ1) (205), and nidogen/entactin, together with proteoglycans such as agrin and perlecan, as well as glycoproteins. The basement membrane of the glomerular capillaries is much thicker (240–370 nm) (163) than the basement membranes in other vascular beds (40–80 nm) (288).

The collagen IV network may be described as the backbone of the basement membrane. Mutations in the collagen chains give rise to severe pathological conditions, the most well-known being Alport’s syndrome (hereditary glomerulonephritis) (14, 170, 195). Depending on the location of the mutation, less severe conditions such as thin glomerular membrane disease may occur (320). The basement membrane contains large amounts of proteoglycans, with mainly heparan sulfate chains attached. These chains may contribute to the selective properties of the barrier (118, 145). However, the importance of the GBM in renal permselectivity has been debated. For a long time, it was considered to be the principal barrier (15, 43, 94, 303). Then in vitro studies of isolated basement membranes suggested that additional components were required to maintain glomerular permselectivity (20, 65). In another study, the inert probe Ficoll and its negatively charged counterpart Ficoll sulfate were used to investigate the charge effect of isolated GBMs (28). There was no difference between the sieving curves of negative and neutral Ficolls. This suggests that the bulk of effective charge density within the glomerular barrier lies in the endothelial or epithelial cell layers. However, the GBM is still an important part of the glomerular barrier, since it accounts for most of the restriction of the fluid flux (65, 73). Moreover, recent data on laminin β2 in patients (350) and knockout mice (132) seem to indicate that the GBM may restrict solute flux as well. In the mice, proteinuria preceded alterations in podocyte morphology, suggesting the GBM to be an important part of the glomerular barrier (132). However, it could not be ruled out that endothelial or epithelial functions were affected by the loss of laminin and normal GBM structure (116).

C. Endothelium

The endothelial cells of the glomerulus have not been well investigated, likely because they are located within the glomerulus and because they are difficult to maintain in culture without altering their phenotype. Glomerular endothelial cells are unusually flattened, with a height around the capillary loops of ~50–150 nm (315). Capillaries with continuous endothelium are the most common type and are typically found in skeletal muscle, cardiac muscle, and skin (288). However, the glomerular capillaries have endothelial cells with a large fenestrated area constituting 20–50% of the entire endothelial surface (40). The unusually high density of fenestrae is thought to
allow high permeability to water and small solutes in the glomerulus (73). A scanning electron micrograph of a mouse glomerulus and a fenestrated capillary is shown in Figure 2.

The fenestrae are ~60 nm in diameter (316), and albumin, which is restricted by the glomerular wall, has a diameter of only 3.6 nm. These measurements have been used to suggest that endothelial cells do not contribute to the permselectivity of the glomerular barrier. However, the endothelial cell coat has charge-selective properties and the barrier probably begins at the endothelial level. For instance, the presence of a diaphragm across the fenestrations in glomerular capillaries has been a matter of debate. Rostgaard and Qvortrup (260) described a high density of fibers in the fenestrae, probably consisting of negatively charged proteoglycans. Other studies support a permselective layer at the level of the endothelium (11, 52, 70, 134, 135, 212, 302). These studies include both morphological (267, 268) and functional data and provide support for the notion that glomerular endothelial cells and their cell coat should be considered in evaluating the permselective properties of the glomerular barrier. A human glomerulus with the endothelial and podocyte cells stained with different markers is shown in Figure 3. Thus the endothelium seems to be an important component of the glomerular barrier, restricting macromolecules despite the presence of numerous large fenestrae. Recently, there has been a renewed interest in the cell surface coat that is produced by, and surrounds, the endothelial cells.

D. The Endothelial Cell Surface Layer

On the luminal side, blood vessel walls are covered with an endothelial cell surface layer (ESL) that is involved in blood coagulation (16, 174), modulation of angiogenesis (39, 96), rheology (62), and capillary barrier function (129, 134, 135, 301). This layer has two components: the glycocalyx, which most commonly refers to the plasma-membrane-bound part of the layer, and the endo-

![FIG. 2. Scanning electron micrograph showing a mouse glomerulus (A) with several capillary loops, capillary lumen, and podocytes with their foot processes. To the right (B) is a fenestrated glomerular capillary with its fenestrated endothelium surrounded by podocyte foot processes. Scale bars: 10 µm (A) and 1 µm (B).](http://www.prv.org)

![FIG. 3. A human glomerulus with podocytes stained green with antibodies against synaptopodin and endothelial cells stained red with *Ulex europaeus agglutinin I* lectin.](http://www.prv.org)
The epithelial cell coat, a larger, more loosely associated part of the layer. Originally described in the 1940s as a thin, noncellular coat (44, 63), the glycocalyx was first visualized by staining with ruthenium red, a cationic dye that binds to negatively charged molecules (178). The ESL is composed of negatively charged glycoproteins, glycosaminoglycans (GAGs), and membrane-associated and secreted proteoglycans, and can be visualized by using different dyes (Fig. 4).

It has been challenging to visualize the glycocalyx and the cell coat because of technical issues (e.g., dehydration and disruption during fixation and staining) and because shear stress and plasma components are important aspects of the in vivo situation. Moreover, associated plasma proteins such as orosomucoid and albumin affect the composition (114, 115, 117) of the endothelial cell coat. Earlier work using different dyes to visualize the glycocalyx indicated a thickness of 50–100 nm (112, 289). However, intravital microscopy studies with fluorescent tracers indicated a thickness of 0.5 μm (339), while other measurements suggested an even thicker layer, up to 1 μm (77). These differences probably reflect the binding of the dyes to the membrane-bound glycocalyx, which is present after fixation, whereas intravital microscopy reveals the thicker ESL, which is present when the surrounding environment is physiological. In two studies (121, 135), intralipid droplets were infused, and the distance from the droplets to the vessel wall was calculated. The results indicated a large ESL of ~200–400 nm. Digestion of proteoglycans with hyaluronidase, heparinase, or chondroitinase reduced the thickness of the ESL (121, 135). Treatment with chondroitinase also increased the fractional clearance of albumin (135). Note that the enzymes used mainly affected the ESL and not the GBM or other structures of the barrier, since the high molecular weights of the enzymes made them remain mostly in the vascular compartment during the experiment (135). Thus the ESL seems to be a rather thick, negatively charged structure that most likely contributes to the high permselectivity of the glomerular wall.

1. **The podocyte glycocalyx**

Podocytes are covered by a glycocalyx containing sulfated molecules such as GAG and sialylated glycosyl conjugates (232). Most studies of the podocyte glycocalyx were done using cationic dyes, which have, for example, shown that heparan sulfate GAGs are present at the slit membrane (261). The major known sialoprotein on podocytes is podocalyxin, a heavily glycosylated protein (151). In rats treated with puromycin to mimic a nephrotic syndrome, the sialic acid content of podocalyxin was lowered, indicating that foot process effacement is linked to a reduced negative charge on the podocyte surface (152). Glomerular epithelial cells also produce proteoglycans such as glypican-1 and syndecan-4 (238). The surface anionic charge on podocytes is essential for maintaining the foot process structure (25). It is also involved in keeping a distance between parietal and visceral epithelial cells, thereby helping to maintain glomerular structure and function (150).

**E. Mesangial Cells**

The main function of the glomerular mesangium is to maintain the structure and function of the glomerular barrier. The mesangium is composed of two entities: mesangial cells and the extracellular matrix. Like smooth muscle cells, mesangial cells have contractile properties (162, 277). Although this contractility might suggest that mesangial cells influence filtration, their overall contribution to permselectivity is probably small. Rather, the contractility may regulate glomerular distensibility in response to pressure.

Mesangial cells produce components of the mesangial matrix, which is thought to provide support for glomerular capillaries. The mesangial matrix consists of col-

![Fig. 4](https://physrev.physiology.org/)

**Fig. 4.** Electron micrographs showing the glomerular barrier, with the capillary lumen above and the urinary space below. The endothelial cell surface coat (ESL) can be visualized with different techniques (121). A: Cupromeronic Blue was used to visualize the charged structures of the barrier. With this technique, the GBM appears to have a homogeneous structure. B: Staining with lanthanum results in higher contrast and shows "bushlike" structures in the fenestrate that extend into the capillary lumen. Scale bars: 100 nm.
lagens (type I, III, IV, and V), laminin, fibronectin, and proteoglycans with both heparan sulfate and chondroitin sulfate chains (189, 215). Mesangial cells secrete several growth factors, including interleukin (IL)-1, platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF) (1, 8, 352). PDGF is a potent mitogen for mesangial cells. Transforming growth factor (TGF)-β has the opposite effect and inhibits cell proliferation; it also stimulates proteoglycan synthesis in both mesangial cells and podocytes in culture.

Mesangial cell proliferation and matrix expansion are seen in several pathological conditions, such as IgA nephritis (143, 165) and diabetic nephropathy (102, 133, 276). As the mesangial matrix expands, it affects the glomerular capillaries by reducing the area available for filtration and may eventually even occlude the capillary lumen. Matrix expansion due to cell proliferation also leads to glomerulosclerosis.

F. Plasma Components

The idea that components of plasma influence the glomerular barrier is not entirely new (164), but it has mainly concerned “permeability factors” that induce proteinuria (98). Several candidates have been suggested, but causal associations have not been demonstrated (34). In other capillary beds, low concentrations of albumin (<1% of normal) increase permeability to water (63, 191). In the kidney, however, the absence of albumin seems to increase glomerular permeability to macromolecules as well (92). Orosomucoid (a plasma protein and acute phase reactant normally present in concentrations between 0.1 and 1.0 g/l in human plasma) has a similar permselective effect in several vascular beds (58, 114, 115), including the kidney (117, 139). The mechanism is not clear, but it may involve fiber matrix interaction (168), endothelial cell production of orosomucoid (299), binding of orosomucoid to endothelial cells (279), or an anti-inflammatory cAMP-mediated receptor activation by orosomucoid (300).

In other vascular beds, plasma proteins increase the thickness of the endothelial cell surface coat (often denoted as glycocalyx) (2). Any sufficiently concentrated protein (such as albumin in normal plasma) would be expected to increase the equilibrium partition coefficient for itself and other macromolecules in glycocalyx and possibly GBM, which in turn would influence glomerular sieving (168) (see sect. vi). Thus there seem to be ample possibilities for plasma proteins, such as orosomucoid, to affect the glomerular barrier.

Figure 5 shows a schematic drawing of the glomerular barrier with focus on the endothelium. Podocytes...
influence endothelial properties by secreting hormones such as vascular endothelial growth factor (VEGF) and ANG I. The glycocalyx is composed of the membrane-bound proteoglycans such as syndecan and glypican. The ESL is a much thicker structure, which also consists of secreted proteoglycans (e.g., versican and perlecan), hyaluronan, and adsorbed plasma proteins (e.g., orosomucoid and albumin).

III. METHODS TO STUDY THE GLOMERULAR BARRIER

Several experimental approaches have been used to unravel the complexities of glomerular permeability, including urinalysis in vivo, micropuncture of single nephrons, isolated perfused kidneys, tissue-uptake techniques, isolated glomeruli, isolated glomerular cells, isolated basement membranes, and artificial membranes. Each approach has merits and drawbacks.

A. Urinalysis In Vivo

In vivo urinalysis is readily available for studies in humans. The use of metabolically inert solutes such as inulins (oligosaccharides derived from plants) is the gold standard for estimating the glomerular filtration rate (GFR) and for analyzing solute sieving.

It might seem more physiological to analyze endogenous proteins (308, 309), but such data are less useful (203) due to the extensive reabsorption, possible degradation, or secretion by tubular cells. Attempts have been made to correct for these limitations, but the accuracy of the corrections is still uncertain (310, 311). Data concerning glomerular size selectivity in humans are mainly based on studies using dextran (3, 106, 243, 244) or Ficoll (26). Human glomerular charge selectivity has been studied using sulfated dextran (105).

Patients with defects in the reabsorption machinery of the proximal tubule (i.e., Fanconi syndrome) excrete the proteins filtered across the glomerular wall. In a series of elegant studies in such patients, modern proteomics approaches such as matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) were used to analyze urine composition (59, 207, 208). The results showed that the megalin-cubulin complex in the proximal tubules was impaired (206), and protein excretion was increased almost 100-fold (208). This value is compatible with a highly selective glomerular barrier (45–48, 68, 73, 116, 317).

B. Micropuncture of Single Nephrons

Micropuncture of single nephrons is an excellent technique for studying pressures, flow, and small-solute transport. For analysis of macromolecules such as albumin, however, certain technical problems emerge. For example, if samples are obtained from the proximal tubules and not from Bowman’s capsule, the urinary concentration of albumin will be underestimated. Adsorption of protein to the surface of the glass pipette may cause further underestimation. In addition, the sampling procedure itself may damage the delicate barrier. In an attempt to circumvent some of these limitations, Tojo and Endou (317) plotted the albumin concentration against the tubular fluid to plasma concentration ratio (TF/P) of inulin (317). Retropolating to a TF/P of 1.0 for inulin, they estimated the sieving coefficient for albumin to be 0.0006. Furthermore, their data show that the albumin concentration is underestimated by one order of magnitude when the TF/P is 2. In contrast, the normal urinary excretion of albumin in humans is <20 mg/day, giving apparent sieving coefficients of >10^-5, reflecting the extensive tubular modification of the urinary content.

C. The Isolated Perfused Kidney

This technique has been used for many years (343) but with several modifications. For example, the kidney can be perfused at normal temperatures (37°C) (30, 180, 257) or at reduced temperatures [4–8°C, cooled isolated perfused kidney (cIPK)] (173, 240). As perfusate, one can use blood (33, 60, 248) or artificial solutions containing albumin (140), dextran (297), or other colloids (38). Tubular modification of the primary urine has been minimized by using chemical agents (222, 228), low temperatures (140, 213, 240, 302), and chemical fixation (51–53). Finally, the kidneys can be perfused with recirculating (33) or single-passage systems (140).

Perfusion with blood is theoretically more physiological but is technically difficult for several reasons, such as the risk for hemolysis, complement activation, and thrombocyte degranulation. Therefore, most researchers interested in glomerular permeability have used artificial solutions based on albumin or other colloids. However, one problem with a blood-free solution is that the maximum amount of oxygen that can be dissolved in water does not meet the metabolic demands of the kidney, as reflected by morphological evidence of renal ischemia (173). Fluorocarbons can be used (91), but such agents might affect the permeability per se. In the warm IPK, renal hypoxia leads to albuminuria that gradually increases over time. The kidneys may be better preserved if the perfusate contains mannitol and amino acids (180, 333). Kidneys perfused at reduced temperatures (8–27°C) do not show ischemic damage (173, 177); nevertheless, albumin clearance increased gradually over time (117).
To study glomerular filtration, one must either inhibit tubular cell activity or use inert tracers that are neither secreted nor reabsorbed in the tubules (30). Tubular cell toxins, such as lysine (150 mM), ammonium chloride (10 mM), chlorochine (0.1 mM), and cytochalasin B (0.02 mM) (228), increase glomerular permeability (211) and cannot be recommended. The cIPK model is most useful for studies of macromolecular transport, since the equivalent pore dimensions are similar in the cIPK model and in vivo (123), a finding reported for warm IPKs as well (30) using dextran as the inert tracer.

D. Tissue-Uptake Techniques

Glomerular function can also be studied by analyzing the tissue uptake of radiolabeled tracers administered in vivo. However, this approach assumes that the filtered tracer is present in the urine, the tubular system, or the tubular cells (19), at least shortly after administration (312, 313). Radioactivity is counted in plasma, urine, and the removed kidneys, allowing for estimates of clearance. Comparisons between experimental groups of animals (12) are hampered by the fact that there may be differences in hemodynamic conditions and in the number of nephrons, and hence differences at the single-nephron level affecting the filtration rate (179, 251). Such differences may affect the clearance of tracers even in the absence of alterations at the membrane level, a phenomenon sometimes neglected, as will be discussed in section VI.

E. The Isolated Glomerulus

Isolated glomeruli have been used to estimate albumin permeability (274), based on osmotically induced changes in glomerular volume. The underlying theory is that solutes exert between 0 and 100% of the theoretical osmotic pressure across membranes, the fraction of the theoretical osmotic pressure being equal to the osmotic reflection coefficient ($\sigma$). Therefore, the authors interpret changes in glomerular volume in terms of alterations in $\sigma_{\text{alb}}$. This is not an adequate term, however, since numerous factors (e.g., cellular contraction) can affect the glomerular volume apart from the permeability of albumin. Moreover, reported $\sigma_{\text{alb}}$ values of 0.5 or less in response to damage (61, 188, 281, 285, 286) are unrealistically low. Such values would imply urinary albumin losses for nephrotic patients in the range of several kilograms of albumin per day ($180 \text{ l/d} \times 40 \text{ g/l} \times 0.5$), far more than is compatible with life. However, the model may still be useful as a diagnostic tool in search for proteinuric factors in serum (42, 282–284, 318). The diffusional permeability of macromolecules has also been studied in isolated glomeruli (64, 65, 81, 82). In certain animals, it has even been possible to microdissect and perfuse single glomeruli for studies of glomerular transport (88).

F. Isolated Glomerular Cells

Cells cannot be used to analyze the properties of the complex glomerular barrier. However, studies in cells are crucial for understanding how it is created and maintained. Glomerular endothelial (13, 23, 99, 233, 272, 273, 298, 324), epithelial (125, 199, 200), and mesangial cells (156, 275) have been cultured and studied in isolation. Human podocytes in culture are shown in Figure 6. Fewer studies have focused on interactions between the different cell types (e.g., mesangial and endothelial cells) (156). Glomerular cells produce molecules that may have endocrine and autocrine functions, such as VEGF (66, 103), and their signaling effects are most likely important for glomerular function (79, 85, 125, 142, 182, 304).

To study the importance and contribution of the different cell types in the glomerulus, methods have been developed to separate the various kinds of cells. Glomeruli are easy to harvest from kidney samples of humans.
and the most commonly used species such as rats and mice. They are readily visualized by light microscopy and may be microdissected from renal tissue. For a more large-scale approach, glomeruli can be obtained by passing renal tissue through stainless steel sieves with specific mesh sizes (13) or by using magnetic beads (305). To single out the different cell types, the glomeruli must be broken and the cells released (e.g., with collagenase or other enzymes). For human kidney tissue, the age of the donor is also of importance for the outcome (99), with the cells from a young donor maintaining their phenotype better. The cells may be cultured and then subcloned, or specific cell sorting may be performed through FACS or Percoll gradients.

Culturing the cells poses different challenges depending on the cell type. Mesangial cells were the first cells of glomerular origin to be cultured. They rapidly overgrow other cells in a coculture and are thus easier to maintain in vitro than the other two cell types (89). It is vital that the cultured cells are properly evaluated. Cells in primary cultures may lose their phenotype and stop expressing cell-specific markers. This has lately been addressed by creating immortalized lines of both human and mouse glomerular endothelial cells (272, 331) and podocytes (200, 201, 270). Still, it is important to characterize the cells for specific markers. Is an endothelial cell without specific markers such as platelet/endothelial cell adhesion molecule 1 still an endothelial cell? Is it relevant to study a podocyte that does not express nephrin and CD2AP?

G. Glomerular Endothelial Cells

Primary cultures of glomerular endothelial cells are difficult to work with. The cellular phenotype is lost after only a few passages, so reliable information can only be gained from low-passage primary cultures. Most of the earlier work on endothelial cells and their properties was done on cells derived from large vessels (e.g., aortic and umbilical vein endothelial cells). Although such studies have yielded important information, there are differences both between species and between different vascular beds. A recent study comparing bovine aortic endothelial cells to glomerular endothelial cells revealed significant transcriptional differences between cells of macro- and microvascular origin (280). To circumvent problems with dedifferentiation, immortalized lines of glomerular endothelial cells were created.

Another important factor is shear stress. Endothelial cells showed marked differences in expression and morphology in the presence and absence of shear stress (196). Nevertheless, cultures of glomerular endothelial cells are an important source of information for cell function. For all studies on glomerular endothelium, it is important to characterize the cells carefully using specific markers (Fig. 7).

H. Proteoglycan and GAG Production

Proteoglycans are versatile molecules found in diverse areas of the body. The hallmark of a proteoglycan is a protein core carrying at least one GAG chain. GAGs are sulfated polysaccharides consisting of repeating disaccharide units of uronic acid (glucuronic acid or iduronic acid) and amino sugar (galactosamine or glucosamine). The GAG moieties of the proteoglycans are divided into groups. Chondroitin sulfate and dermatan sulfate contain galactosamine and are called glucosaminoglycans. Heparin and heparan sulfate contain glucosamine and are therefore called glucosaminoglycans.

![FIG. 7. Human glomerular endothelial cells in primary culture. A: light microscopic image of normal cells. B: cells labeled with Dil-Ac-LDL, an endothelial cell-specific agent. C: cells labeled with an endothelial cell-specific lectin from Ulex europaeus agglutinin I.](image-url)
Several enzymes regulate GAG chain synthesis and sulfatation. The amount of attached sulfate groups determines how negatively charged the GAG will be. The galactosaminoglycans (chondroitin sulfate and dermatan sulfate) are modified by sulfotransferases (4-O or 6-O), which add sulfate groups to the carbohydrate chain, and 2-O-sulfotransferase, which adds sulfate groups to the uronic acid residue. The N-deacetylase N-sulfotransferase enzyme family is involved in glucosaminoglycan (heparin and heparin sulfate) sulfatation. There are also several O-transferases that add sulfate groups to the glucuronic acid moiety. In addition to the different GAG chains, there are also several proteoglycan core proteins (Fig. 8).

In the glomerulus, the most described proteoglycans are perlecan (131) and agrin (110, 239), both expressed in the GBM together with another proteoglycan, collagen VIII (113, 202). Perlecan carries heparan sulfate and sometimes chondroitin sulfate, but agrin carries only heparan sulfate. These molecules contribute to the negative charge of the GBM and may thus contribute to the permselectivity of the glomerular barrier. However, mice lacking perlecan heparan sulfate chains do not develop proteinuria or any other renal defects (258). This may be due to compensatory mechanisms, but more likely it further strengthens the findings that the GBM alone cannot be responsible for the permselective properties of the glomerular barrier. Proteoglycans are present on the surface of endothelial cells and podocytes and in the mesangial cell matrix as well. Cultured immortalized human podocytes express both mRNA and protein for syndecan-1, versican, and perlecan (24). The negative charges on podocytes are thought to contribute to charge selectivity, podocyte stability, and signaling. Bovine glomerular endothelial cells synthesize perlecan, a synthesis that may be altered by treatment with TGF-β (148) or glucose (109). Biglycan is expressed by glomerular endothelial cells in tissue sections, and human glomerular endothelial cells in culture express several proteoglycans (syndecan, versican, glypicanc, and perlecan) along with their regulatory enzymes (sulfotransferases) (23). In addition, treatment with puromycin aminonucleoside downregulated proteoglycan expression in glomerular endothelial cells and induced a nephrotic syndrome in rats (24).

I. Isolated GBMs

Isolated GBM has been used extensively to study the transport of fluid and water-soluble solutes (65, 82, 93, 130). These studies have been of great importance for our understanding of sieving across gels or fiber matrices. In isolated GBM, charge selectivity has been studied using dextrans (32) and Ficolls (28), and the effect of plasma proteins on the selectivity of the GBM has been analyzed (168). When extrapolating data from the isolated basement membranes to the GBM in vivo, one must consider the possibility that certain components may have been lost during the isolation procedure (28). Moreover, the hydraulic conductivity and the sieving properties of isolated GBM can be modulated by elevating the hydrostatic pressure on both sides of the membrane (253). There are, however, strong arguments to suggest that the GBM has similar properties in vivo and in vitro. The hydraulic conductance of isolated GBM accounts for most of the fluid restriction of the intact glomerular barrier (65, 73). Therefore, it seems safe to conclude that the GBM normally restricts water transport but only marginally restricts the passage of solutes (28).

J. Artificial Membranes

Synthetic membranes of various types have been used to examine the solute and membrane properties that govern the transport of proteins and other macromolecular solutes. Results for pores or microchannels of precisely controlled dimensions have been reviewed (67, 69). The desire to better understand and mimic materials such as GBM has motivated several studies of transport through hydrogels, including agarose and agarose-dextran composites (138, 141, 158 –160, 167). Such studies are gradually refining our understanding of the physics of
hindered transport through networks of cross-linked polymers with fluid-filled interstices.

IV. SOLUTES USED TO STUDY GLOMERULAR PERMEABILITY

A. Theoretically Ideal Solutes

The ideal solute for studies of transport is a solid inert sphere that does not affect the organism in any way and is not metabolized during the observation period. For studies of glomerular charge selectivity, solid spheres should carry a known net charge without distorting molecular size or shape and should remain metabolically inert. To assess the effect of shape, the solid molecules should be made ellipsoid in a controlled and stable manner. Unfortunately, the ideal solute does not exist. Therefore, in interpreting the results of clearance studies with "real world" solutes, their nonideal properties must be taken into account. At present, Ficoll seems to be closest to the "ideal" solute (see sect. VI).

B. Proteins

Endogenous proteins are highly relevant to study, but they deviate from the ideal solute in many respects. By definition, proteins are not inert, are never solid, seldom have spherical shapes, are often compressible, and are made of amino acids with a neutral, negative, or positive charge, giving them a certain pH-sensitive net charge. To complicate things further, charges may be expressed on the surface or not, and the protein core may carry GAG chains. The most common issue with respect to glomerular filtration is, however, the tubular handling of proteins. The most reliable estimates of the glomerular sieving of proteins come from studies in which reabsorption is controlled.

Estimated sieving coefficients for albumin (\(\Theta_{ab}\)) from studies controlled for tubular modification of the urine are shown in Table 1. Micropuncture of rat proximal tubules (317) gives values for \(\Theta_{ab}\) (assuming a serum albumin concentration of 30 g/l) that are 10-fold higher than estimates from patients with Fanconi (Dents) syndrome (i.e., \(8 \times 10^{-5}\)) (208). The latter value represents a lower limit for the sieving coefficient, since there still may be a residual reabsorption of albumin. Lower values (\(3 \times 10^{-4}\)) were obtained in vivo in rats in which tubular reabsorption was (partially? Ref. 314) inhibited with lysine (310). The \(\Theta_{ab}\) values from cIPK (\(8^\circ C\)) are two- to threefold higher (122, 123, 176, 301, 302), but still close to 0.001. In contrast, estimates from IPK at \(37^\circ C\) are 10 times higher (225, 228) and increase further, to 0.08, after treatment with a tubular cell inhibitor (228). The latter value is not compatible with the notion of a highly selective glomerular barrier and gave rise to the so-called albumin retrieval hypothesis (see sect. vii).

Glomerular sieving data for other proteins during controlled tubular cell activity are scarce. In vivo, lysine has been administered to produce proteinuria by inhibiting tubular cells (310). However, the albumin sieving coefficient calculated from such data is only half of that obtained with micropuncture (317) or tissue-uptake techniques (19, 179) or from patients with Fanconi syndrome (209) (Table 1). Indeed, lysine has variable and incomplete effects, and its mechanism of action is still unclear (314). Therefore, the sieving data for \(\beta2\)-microglobulin, orosomucoid, IgG, and \(\alpha2\)-macroglobin are likely to be underestimated as well (310). To circumvent some of the problems with administration of lysine, Lund et al. (179) used a tissue-uptake technique to estimate glomerular sieving of certain proteins. Although attractive, this technique is an indirect approach based on certain assumptions, as described in detail elsewhere (312). The clearance data from tissue-uptake studies (18, 19, 179) are similar to those derived from measurements using neutral Ficoll in vivo (26, 219). These values in turn are similar to those from cIPK studies (122, 123, 176, 301, 302) (Table 2).

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<th>TABLE 1. Reported sieving coefficients for albumin in studies with “controlled” tubular modification</th>
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C. Dextran Polymers

Dextran has been extensively used for transport studies in the kidney and other organs (250). It is an α-1,6-glucose-linked polymer with a few short side branches that behave as a flexible hydrated random coil rather than as a solid sphere. In the kidneys, dextran was used 30 years ago in the classical studies of glomerular size and charge selectivity (17, 36, 45–48). However, pore dimensions based on neutral dextran sieving data were overestimated (219), owing in part to dextran’s flexibility (218). Thus, compared with a rigid solute such as Ficoll or a globular protein, the sieving coefficient of dextran may be an order of magnitude too high (219).

Sulfated dextran raises other concerns. Notably, it does not appear to be fully inert. Some batches of dextran sulfate seem to bind to plasma proteins, and certain tubular cells may bind and incorporate the solute. As a result of either effect, the concentration in urine will be underestimated, and charge selectivity will be overestimated. Indeed, the glomerular charge density estimated from studies of dextran sulfate is ~120–170 meq/l (76) but only 30–50 meq/l in studies of proteins (176, 302, 346, 347) or Ficoll (123, 134, 301). Other elongated and/or flexible molecules that behave like dextran have sieving coefficients larger than expected from their Stokes-Einstein radii, such as hyaluronan (214) and bikunin (175, 214). Thus there are good reasons to avoid dextran for studies of transport across biological membranes.

D. Ficoll Polymers

Ficolls are neutral, heavily cross-linked, sucrose-epichlorohydrin copolymers. The molecules behave as rigid hydrated spheres and can be labeled with isotopes (26) or fluorescent dyes (213). The normal molecular Stokes-Einstein radius for Ficoll 70 is 10–70 Å (213). Ficolls are inert and do not seem to be reabsorbed, secreted, or degraded in kidneys in vivo. Indeed, neutral Ficoll is close to the ideal solute for studies of transport across biological and artificial membranes. Ficolls, including sulfated Ficoll, do not bind to plasma proteins (28). Whereas sulfated Ficoll seems to behave according to hydrodynamic laws (28), there have been reports of anomalous behavior of carboxymethylated Ficoll (10, 107). The mechanism responsible for the high clearance for carboxymethylated Ficoll remains to be elucidated (10, 107). The sieving coefficients for Ficoll from intact rats in vivo (219) and cIPK in mice (134) are plotted against their molecular Stokes-Einstein radius in Figure 9.

E. Proteins Versus Synthetic Polymers

Some investigators suggest that proteins behave differently from Ficoll in their transport (264) across biological and artificial membranes (335). This view is based on three arguments: 1) that Ficoll and protein glomerular sieving data differ; 2) that Ficoll may not be uncharged; and 3) that the Ficoll solute radius may change in response to changes in ionic strength.
Concerning the first argument, the glomerular sieving data depend on the Peclet number (see sect. vi), and it may be difficult to compare observations obtained under different experimental conditions. It would be more relevant to determine if neutral spherical proteins differ from Ficoll under controlled conditions, such as using artificial membranes or isolated GBM. Indeed, such studies suggest that proteins and Ficoll are rather similar (28, 71, 136, 141). Furthermore, neutral proteins seem to behave similarly to Ficoll in IPKs if studied under similar and standardized conditions (Fig. 10).

The second argument, that Ficoll may be charged (335), is easily addressed. FITC-Ficoll moves slowly during routine serum protein electrophoresis and behaves as a neutral solute even though FITC has a charge of $-1$ (cited in text in Ref. 301). Concerning the third argument, HPLC gel filtration of sharp fractions of Ficoll at normal, low, and high ionic strengths (as used in Refs. 301, 302) showed that the Ficoll Stokes-Einstein radius was rather stable. It was not significantly altered by reducing the ionic strength to 20 mM ($0.02 \pm 0.18 \text{Å, } n = 10$) or by increasing it to 520 mM ($0.61 \pm 0.26 \text{Å, } n = 10$, not significant) (B. Haraldsson and J. Nyström, unpublished observation).

We conclude that Ficoll in many respects behaves as an ideal solute. Its transport across artificial and biologi-
cal membranes is quite similar to that of uncharged spherical proteins.

V. SOLUTE PROPERTIES THAT AFFECT TRANSGLOMERULAR PASSAGE

A. Molecular Size

Solutes are retarded in their passage across the glomerular barrier depending on their molecular size. This is evident from a large number of human and experimental studies using dextran (47), Ficoll (26, 213, 219), and proteins (179). Glomerular size selectivity can be expressed in terms of several different theoretical models. According to the two-pore theory, the equivalent small pore radius is close to 45–50 Å (26, 212, 213), the large pore radius is close to 80–100 Å (176, 212, 213, 310), and the large pore pathway represents 0.003 or less of the total hydraulic conductance (213). For the lognormal pore distribution model, the mean pore size seems to be close to 35–45 Å, with a distribution parameter of 1.3–1.1; the lower pore radius value is then combined with the broader distribution parameter (26, 213, 219) (Fig. 9). Finally, glomerular size selectivity has been described by the charged-fiber model, according to which the fiber volume is 6–7% (52, 134) (Fig. 9). All these theoretical models describe the sieving of solutes more or less to the same degree. However, they evolved over time, and only the latter model incorporates both size and charge effects.

As mentioned above, dextrans give falsely high values for the small pore radius, which typically is estimated to be 50–60 Å (37, 242, 263), almost 10 Å greater than the “true” equivalent pore radius. However, pore size can be markedly underestimated as well. Reports based on protein analysis in vivo suggest a small pore radius of 30 Å or less (310). In the latter study, the work of Remuzzi et al. (242) was cited erroneously as supporting such a small pore size. The underestimation of the pore size reflects the limitations of in vivo protein analysis, even in the presence of tubular inhibition with lysine (310), which is only partial (314). Indeed, using a tissue-uptake technique, authors from the same group estimated the pore radius to be 38 Å (179). Naturally, the estimates of pore radii depend on the biological conditions during the experiment, and occasionally low pore radii are found even with Ficoll (217).

The studies above indicate that, for neutral solutes, the sieving coefficient decreases as molecular size increases. This relationship can be described adequately with at least four different transport theories: the two-pore model, the lognormal pore distribution model (with or without shunt), (neutral) fiber matrix theory, and the negatively charged fiber model (136, 137). As mentioned above, the negatively charged fiber model incorporates both size and charge selectivity (52) and it fits well with biological data (Fig. 9; see also Fig. 1 in Ref. 134). The equations are well developed for the partition coefficient, but the model is less mature in terms of estimations of the reflection coefficients (see Ref. 351 and below for more information). The size selectivity seen in Figure 9 for Ficoll is from three different studies from rats in vivo (219), rat cIPK (301), and mouse cIPK (134). Furthermore, Figure 10 shows that neutral proteins behave as neutral Ficoll (the two curves are from Refs. 134, 301).

In Table 2, the sieving coefficients for various neutral proteins plotted in Figure 10 are given together with the reference. There are two values for “neutral” albumin, one obtained with a tissue-uptake technique (19) and the other with IPKs (228). The latter value is likely to be an underestimate, since tubular cell activities were not completely blocked. The charge-modified albumin may form dimers, spontaneously regain its negative charge, and bind to tissue structures, as described in detail elsewhere (108). Indeed, values that are only 20% of those of Reference 228 have been reported for neutral albumin (179).

From the latter study, the value for κ-dimer is obtained together with data for neutral myoglobin. Similar data for neutral horseradish peroxidase (HRP) have been reported in some studies (246, 302, 348), and 30–50% lower values in others (179, 222). Finally, data have been reported for IgG (18), lactate dehydrogenase-5 (176), and slightly cationic myoglobin (348). The protein sieving data of Lund et al. (314) are systematically lower than expected. As described above, the inhibition of tubular uptake by lysine infusion seems to be incomplete and variable (314), leading to underestimations of the transglomerular passage of proteins. We conclude that neutral Ficoll and neutral proteins behave similarly in their passage across the highly size-selective glomerular barrier.

B. Molecular Charge

The first experimental evidence of capillary charge selectivity was reported in 1969 by Areekul, who used neutral and sulfated dextran (7). Several now-classical studies suggested strong charge selectivity in the kidney, with anionic macromolecules being more retarded than their neutral counterparts of similar sizes (35, 36, 45–48, 76). Similar effects were found using anionic, cationic, and neutral HRP (246). This view prevailed for decades. In 1991, however, the use of dextran sulfate was called into question (306). This led to a series of critical papers questioning the very existence of glomerular charge selectivity.

1. Is there any glomerular charge selectivity?

Over the past decade, a large number of studies, mainly from one laboratory, have suggested that glomer-
ular charge selectivity does not exist at all (264). In arguing against a glomerular charge barrier, Comper and colleagues make several assertions: 1) that all studies using dextran sulfate are invalid, due to problems with desulfation in the urine (57), binding to plasma proteins, glomerular cell uptake, and binding to GBM (306, 340, 341); 2) that their own data on the glomerular clearance of neutral and anionic HRP (aHRP) (222) and of neutral and charge-modified albumin (228) provide evidence against a charge barrier (264); 3) that albumin is filtered in large quantities across the glomerular wall and reabsorbed in intact form (228); 4) that proteins are degraded in the urine and excreted as small peptides not detected by most routine techniques (223, 227); 5) that anionic dextrans and Ficolls behave anomalously when filtered across glomerular walls (10, 41, 107); and 6) that evidence of charge selectivity is artifactual, due to limitations of the investigative techniques, mainly cIPK (264).

Are any of these arguments valid? Let us start with the questions regarding dextran sulfate. Indeed, dextran sulfate has been shown to bind to fibronectin (210) and to plasma proteins, depending on the molecular weight of the dextran molecules (105). In the latter study, after measurement and correction of such effects, Guasch et al. (105) still found evidence of a negative charge barrier. The data on cellular uptake and binding of dextran sulfate by glomerular cells and its subsequent desulfation were reviewed recently in detail (73). In brief, the cellular processing adduced by Comper and co-workers (266) is rapidly saturable and therefore would not influence steady-state clearance data, such as those reported by Guasch et al. (105). We conclude that the use of dextran sulfate overestimates the charge density of the glomerular barrier, but the data are qualitatively correct.

Next, let us examine the second argument, that the data on neutral HRP (nHRP), aHRP, and albumin provide evidence against a negative charge barrier. The fractional clearance of nHRP was 2.4 times higher than that of aHRP under control conditions (224), a ratio identical to that reported by Sörensson et al. (302) but less than the ratio of 7 (348) or 9 (246) reported in earlier studies. Adding 150 mM lysine (n = 4) or 10 mM NH₄Cl (n = 4) to the perfusate elevated the fractional clearance of nHRP by 70% and of aHRP by 170%, giving a residual charge selectivity ratio of 1.6 (224). Also for albumin, the original data of Osicka et al. (228) do suggest charge selectivity; in the IPK perfused at 37°C, the fractional clearance was 0.0075 for native albumin and 0.0330 for neutral albumin (228). The clearance of native albumin is 5–10 times higher than values reported for cIPK or in vivo with controlled tubular reabsorption (Table 1). Similar levels of clearance were reported for neutral albumin by Bertolatus and Hunsicker using a tissue-uptake technique (19), but their clearance ratio was 43. After treatment with 150 mM lysine, fractional clearance increased 3-fold for neutral albumin and 10-fold for anionic albumin (228). The fact that the tubular cell inhibitors raised the clearance for the neutral solutes and for the anionic proteins suggests toxic effects on glomerular size selectivity. Indeed, toxic effects on glomerular size and charge selectivity were found by Ohlson et al. (213), which seriously questions the validity of the original interpretations (222, 224, 228).

The third argument, that albumin is filtered in large quantities and reabsorbed in intact form, is dealt with below (see sect. vi). It seems safe to conclude that there are ample experimental data to refute the hypothesis.

The fourth argument is that proteins such as albumin are degraded (223, 227), causing protein excretion to be underestimated (100). The validity of this argument has been tested (209) and is discussed in detail in section vi.

The fifth argument is that anionic dextrans and Ficolls behave anomalously when filtered across glomerular walls. The properties of Ficoll and dextran have been discussed earlier in this review. There also have been reports of anomalous behavior of dextran sulfate (41), mainly suggested to be due to increased desulfation in diabetic animals. For charge-modified anionic Ficoll, the clearance is reported to be even more anomalous (107). Thus carboxymethylated Ficoll and neutral Ficoll have similar clearances for solutes with a Stokes-Einstein radius <50 Å; however, for larger molecules, the sieving coefficients differed by more than 100-fold (at 75 Å) (107). Indeed, similar findings of facilitated transport of carboxymethylated Ficoll were reported over a broad molecular size range (15–80 Å) (10). Both papers conclude that anionic Ficoll is unsuitable for studies of solute transport (10, 107). At least two important questions remain unresolved. First, is there active tubular secretion of carboxymethylated Ficoll? Second, is the anomalous behavior specific for carboxymethylated Ficoll or does it hold also for the Ficoll sulfate used in studies of the GBM (28)? Both these question are crucial, since Ficoll sulfate actually seems to behave quite like neutral Ficoll apart from the net charge (28).

In the sixth argument, all data supporting the concept of a negative charge barrier are dismissed as simply being flawed (107, 264). Still, the fractional clearance for albumin at 37°C reported by Ohlson et al. (213) is close to the value of 0.0075 reported by Comper et al. (228) during control situations in the absence of tubular inhibitors. However, the evidence for glomerular charge selectivity does not rest solely on experiments with the cIPK, comparing neutral Ficoll and anionic proteins, as suggested by some authors (10, 107, 264, 335). In Table 2 and Figure 10, data from several experiments are presented for neutral and anionic solutes together with sieving data for neutral Ficoll. The charge-selective effect is obvious, even though the observations were obtained under different conditions. We conclude that the evidence against a negative glomerular charge barrier is weak at best. Let us there-
fore review recent data regarding glomerular charge selectivity.

2. Current understanding of glomerular charge selectivity

Figure 10 illustrates molecular sieving as a function of Stokes-Einstein radius for neutral Ficoll and for several neutral and anionic proteins. The data were from several studies using a variety of techniques, but with more or less controlled tubular activity. It is evident that the neutral proteins are close to the Ficoll sieving curve, reflecting similar properties for neutral proteins and Ficoll. This is in line with data obtained during highly controlled conditions in artificial membranes or isolated GBM (28, 73). The sieving coefficients are considerably lower for all anionic proteins than for neutral proteins, providing strong support for glomerular charge selectivity. Thus, irrespective of theoretical modeling, the net charge of a molecule dramatically affects its transport. In Table 2, sieving data for several neutral and anionic proteins are shown.

Please note that, before correction, the raw data of Comper and co-workers (222, 228) actually support the notion of charge selectivity (Table 2). The authors reached other conclusions based on the effect of the tubular inhibitors, which reduced the clearance ratios (neutral over anionic) for albumin and HRP (222, 228). There are, however, reasons to believe that the agents affect glomerular permeability per se (213). Indeed, the reported dextran sieving values cover a rather narrow range of molecular radii, and the sieving coefficients are somewhat larger than reported for intact animals (47). This is unexpected because perfused kidneys normally have a lower area available for diffusion, which reduces the sieving coefficient for small and intermediate-sized solutes (123). On the other hand, inhibiting tubular cell activity by reducing the temperature to 4–8°C lowers the urine-to-plasma concentration ratio for Cr-EDTA from 20–100 to 1.1–1.2 without significantly altering glomerular charge and size selectivity (213).

The concept of glomerular charge selectivity has been experimentally tested by altering the ionic strength and pH of the perfusate. Sörensson et al. (302) found that reducing the ionic strength from 152 to 34 mM lowered the sieving coefficient (θ) for aHRP by 40%; θ for nHRP was reduced by 15% (302). Similar effects were found for other anionic proteins, including orosomucoid and albumin (301). Moreover, reducing the pH of the perfusate increased the sieving coefficient significantly, in relation to the diminished net negative charge of the protein, and pretreatment with protamine had a similar effect (53), consistent with previous observations (149, 394). Protamine has prominent effects on the intact glomerular barrier but not on isolated GBM (64), suggesting that glomerular cells have a key role in charge selectivity (65). Indeed, our current understanding of the GBM indicates that it has little charge selectivity (28).

Attempts have been made to alter the glomerular charge barrier by using enzymes to digest GAGs (4, 101, 145, 256). Heparan sulfate proteoglycans are major components of the GBM (255); in particular, perlecan (197, 258) and agrin (161, 255) have been reported to affect glomerular permeability. The endothelial cell surface coat (339) also contains GAGs. Heparanase, chondroitinase, and hyaluronidase may increase the sieving coefficient for albumin by reducing the charge density (134) and reducing the thickness of the endothelial cell surface coat (135). In a recent report, mice with adriamycin-induced nephrosis expressed heparinase in their glomeruli, which the control mice did not (161). We conclude that the data obtained with various GAG-degrading enzymes support the notion of glomerular charge selectivity.

Let us use one of the theoretical models to predict the glomerular sieving coefficient for solutes of different molecular size and charge. As mentioned below, all current models have limitations. In a recent paper, a heterogeneous charged fiber model was used to analyze the beneficial effects of orosomucoid on rats with puromycin-induced nephrotic syndrome (122). In Figure 11, the results of such an analysis are presented as a plot of sieving coefficient versus Stokes-Einstein radius. Curves are shown for neutral solutes, slightly positively or negatively charged solutes, and solutes with strong negative charge densities similar to that of serum albumin. Naturally, these curves represent an oversimplification of the glomerular barrier, due to the limitations of current transport equations for a charged fibrous network. There is, however, a reasonable fit between the theoretical curves and the biological data presented in Figure 9.

C. Configuration

The effect of molecular shape on sieving was recognized early by Rennke and Venkatachalam (247), who noted a sevenfold greater sieving coefficient for dextran than for aHRP. It was later found that dextran is less suitable for studies of permeability, since it behaves as a flexible random coil (218) (see sect. iv). The effect of solute shape is not, however, limited to dextran (72). The plasma protein bikunin is an extremely elongated anionic protein with a diffusion constant (and hence a Stokes-Einstein radius) close to that of albumin (290). However, bikunin crosses the glomerular barrier 80 times faster than albumin, despite similarities in molecular size and charge (175). Furthermore, the sieving coefficient for the GAG hyaluronan was three times that of bikunin and 240 times that of albumin despite similarities in size and...
charge (214). In the latter study, the frictional ratios for anionic solutes were related to their “apparent neutral molecular radii” (214). The frictional ratio is 1.0 for a sphere (like Ficoll) and increases with the degree of elongation (1.3 for albumin, 1.8 for bikunin, and 2.3 for hyaluronan). As calculated by Ohlson et al. (214), the apparent neutral molecular radii for the four solutes are, respectively, 35.5, 55.1, 33.0, and 23.5 Å. We conclude that molecular configuration substantially affects solute transport across the glomerular barrier if the solutes are elongated or random coils (71, 73, 214, 247). However, smaller deviations from the ideal sphere shape do not seem to affect transport to any significant degree (28).

D. Relative Importance of Size and Charge Selectivity

Size selectivity is most significant for neutral solutes in the steep portion of the sieving curve (i.e., Stokes-Einstein radius of 30–50 Å; see solid curve in Fig. 11). In that part of the curve, the sieving coefficient falls by one order of magnitude for every 10-Å increase in molecular radius. For neutral solutes up to the SE-radius of 30 Å, the sieving coefficient only falls from 1.0 to 0.1. Also, increasing the molecular radius from 50 to 70 Å reduces the sieving coefficient by a factor of five (Fig. 11). The molecular radius interval between 30 and 40 Å is also where charge selectivity is greatest. Thus a solute charge density similar to that of albumin reduces the sieving coefficient for those solutes to 5–10% of that for a neutral solute.

The charge effect will depend on the charge density of the solute and will occur over the entire molecular size range. The molecular configuration may, however, overrule the effects of size and charge, and elongated solutes of the size and charge of albumin may have sieving coefficients between 0.1 and 1.0 (214). Therefore, to predict the transglomerular passage of a solute, one must know its molecular size (diffusion constant, i.e., Stokes-Einstein radius), its net charge (or charge density), and its configurations, as revealed, for example, by its frictional ratio (214). For solutes that are not random coils or markedly elongated, shape does not significantly affect their transport (28). The sieving coefficients presented in Figure 11 are therefore reasonably accurate for most solutes and predict the effects of molecular size and charge.

VI. PHYSICAL PRINCIPLES AND THEORETICAL MODELS

In reviewing the physical principles that govern the interpretation of data on the glomerular permeability to proteins and other macromolecules, our emphasis is on factors that influence the sieving coefficient of a given
molecule. For ultrafiltration processes in general, the sieving coefficient is the concentration in the filtrate divided by that in the retentate; for the glomerulus, it is usually defined as the concentration in Bowman’s space divided by that in arterial plasma. The sieving coefficient of a solute is determined by multiple factors: the intrinsic selectivity of the membrane based on solute size, charge, and shape; the filtration rate of water; solute concentrations (if the retentate is not dilute); and, for a multilayer barrier such as the glomerular capillary wall, the arrangement of the layers.

In general, one must distinguish between the concentration adjacent to the upstream surface of an ultrafiltration membrane and the average concentration in the retentate. In any sieving process, the concentration of rejected molecules tends to increase next to the upstream membrane surface, a phenomenon called concentration polarization. This increase drives the diffusion of rejected solutes back into the bulk retentate. In industrial ultrafiltration, concentration polarization often leads to a marked reduction in water filtration rates, due to the osmotic effects of the retained solute and/or membrane fouling (349). However, an analysis based on the conditions in glomerular capillaries indicates that the effects there are negligible, because of the small capillary dimensions, moderate filtrate velocities, and mixing effects of red blood cells (75). Accordingly, concentration polarization in the capillary lumen will not be considered here, although an analogous phenomenon within a multilayer membrane will be discussed.

Another key distinction involving retentate concentrations concerns their variations along the length of a permeable tube, such as a glomerular capillary. As plasma moves from the afferent to the efferent end, the concentration of a selectively retained solute must increase because of the proportionately greater removal of water; the greater the filtration fraction of water and the smaller the sieving coefficient, the larger the increase. Such axial variations in concentration have been incorporated into glomerular filtration models since the early 1970s (48, 72, 74, 78), and they have been important for understanding the effects of plasma flow rate and other hemodynamic variables on single-nephron GFR and sieving coefficients. Thus a complete model of the glomerular filtration process must include mass balance equations that describe concentration variations along a capillary. Nonetheless, we have chosen not to discuss axial concentration variations in detail and to focus instead on the determinants of the local sieving coefficient (i.e., the filtrate-to-plasma concentration ratio at a given position along a capillary). Those local factors are most crucial for understanding barrier selectivity and are also most controversial.

A. Hindered Transport and Membrane Size Selectivity

The defining feature of an ultrafiltration membrane is that it can sieve macromolecules on the basis of molecular size. Solutes rejected in ultrafiltration have molecular masses of ~1–1,000 kDa. However, the linear dimensions of a molecule are more directly relevant to its ability to pass through a small pore. The most common such measure of molecular size is the Stokes-Einstein radius \( r_s \), which is related to the diffusivity in bulk solution \( D_s \) as

\[
 r_s = \frac{k_B T}{6\pi \mu D_s}
\]

where \( k_B \) is Boltzmann’s constant, \( T \) is temperature, and \( \mu \) is the viscosity of the solvent. This is the radius of a rigid sphere that would have the given diffusivity. For water at 37°C, a molecule with \( r_s = 1.0 \) nm has a diffusivity of \( D_s = 3.3 \times 10^{-10} \text{m}^2/\text{s} \). For the glomerulus, the range of sizes of most interest for sieving is 2 ≤ \( r_s \) ≤ 6 nm (as discussed in sect. iv), with \( r_s = 3.6 \) nm for serum albumin.

The local solute flux \( (N) \) in a porous or fibrous membrane can be written as

\[
 N = -K_d D_c \frac{dC}{dx} + K_c v C 
\]

where \( C \) is concentration, \( x \) is position, and \( v \) is fluid velocity. This is simply Fick’s first law with a term added for convection (bulk flow) and allowances made for hindrances to solute movement caused by the membrane. The coefficients \( K_d \) and \( K_c \) are hindrance factors that describe the effects of the membrane on solute diffusion and convection, respectively. Thus the apparent diffusivity within the membrane is \( K_d D_c \), and the solute velocity due to bulk flow is \( K_c v \). When Equation 2 is applied to membranes with discrete pores, \( N, v, \) and \( C \) are interpreted as averages over the pore cross-section; for materials of more complex structure (e.g., arrays of fibers with fluid-filled interstices), \( N \) and \( v \) are based on total surface area and \( C \) is usually based on total volume (fluid plus solid). If \( C \) is in molar units \((\text{mol/m}^3)\), then \( N \) has the units \( \text{mol·m}^{-2}·\text{s}^{-1} \). Alternatively, mass units can be used for \( C \) and \( N \).

The basis for membrane size-selectivity is described by theories that predict the values of \( K_d \) and \( K_c \) from \( r_s \) and the quantities that define the membrane nanostructure. A thorough discussion of such theories is beyond the scope of this review, but an introduction to the main ideas will make the subsequent material on sieving more understandable. In general, theories for hindered transport are based on a combination of hydrodynamic and steric considerations. A macromolecule in solution behaves in part
as a hydrodynamic particle, and the presence of fixed objects (e.g., pore walls or membrane fibers) tends to increase the hydrodynamic drag on a particle and thereby reduce its mobility (lowering $K_c$). Interactions between the particle and the wall (or fiber) are mediated by viscous stresses and pressure variations in the fluid. Such hydrodynamic interactions also cause the velocity of a freely suspended particle to deviate from the mean fluid velocity (affecting $K_c$). The finite size of the particle limits the positions that its center can occupy, bringing steric considerations into the calculation of the hindrance factors.

The theory of hindered transport is most completely developed for the special case of spherical molecules in long cylindrical or slit-shaped pores; diffusion and convection in fibrous media are less completely understood. The theory for uniform pores, originating with the work of Pappenheimer, Renkin, and co-workers more than 50 years ago (230, 245), has been reviewed elsewhere (67, 69). Included in those reviews are examples of results from experiments using membrane pores or microfabricated channels of carefully controlled size, which generally confirm the theoretical predictions. The predictions for cylindrical pores are shown in Figure 12, where the hindrance factors are plotted as a function of relative solute size ($\lambda = r_p/r_p$, where $r_p$ is pore radius). Reflecting the progressively greater hindrances to diffusion as relative solute size increases, $K_d$ declines monotonically from unity to zero. The intrapore diffusivity reaches half of the free-solution value (i.e., $K_d = 0.50$) when $\lambda = 0.24$. In other words, diffusion is severely hindered well before the solute becomes a tight fit in the pore.

The pattern for convection is more complicated, in that $K_c$ first increases and then decreases as solute size increases. The initial increase is due to the fact that the finite size of the particle excludes its center from the layer of fluid next to the pore wall, and therefore prevents it from experiencing the most slowly moving fluid. Eventually, though, the hydrodynamic retardation due to the pore wall dominates, and $K_c$ declines. Note that convection tends to be less hindered than diffusion, such that $K_c/K_d \geq 1$, with that ratio increasing with relative solute size. A large value of $K_c/K_d$ tends to aid sieving, as will be seen. The functions $H(\lambda)$, $W(\lambda)$, and $\Phi(\lambda)$, which are also shown in Figure 12, will be discussed shortly.

Although the results in Figure 12 are for cylindrical pores, the qualitative behavior of $K_d$ and $K_c$, with increasing molecular size is similar in slit pores (flat-wall channels) and even in fibrous media, where the transport paths are more tortuous. Only one aspect of those results is unique to cylindrical pores, the convective hindrance of a solute that nearly fills the pore ($\lambda \rightarrow 1$). Whereas $K_c \rightarrow 0$ in that limit for cylindrical pores, for slits and other pore shapes $K_c \rightarrow 0$ for large solutes (67). The peculiar aspect of a tightly fitting sphere in a cylindrical pore is that it completely occludes the channel. With fluid unable to bypass it, the sphere acts as a piston and moves at the mean fluid velocity, rather than becoming immobile. Hence, $K_c \rightarrow 1$ instead of 0.

There have been many applications of pore theory to the glomerulus, as reviewed previously (181). Fiber-matrix theory is less completely developed. For hindered transport through fibrous media, noteworthy recent studies include a discussion of diffusion in randomly oriented fiber matrices (235) and a calculation of the osmotic reflection coefficient for flow along the axes of parallel fibers (351).

### B. Sieving in Homogeneous Membranes

The experimental measure of membrane selectivity in ultrafiltration is the sieving coefficient ($\Theta$). This section begins by relating $\Theta$ to the hindrance factors just discussed, and then illustrates how sieving is influenced also by the operating conditions of the ultrafiltration process. This initial discussion is for a homogeneous or single-layer membrane; multilayer barriers are considered later.

Suppose that a membrane consists of a single layer of porous or fibrous material, extending from $x = 0$ to $x = L$. The concentrations in the retentate and filtrate are denoted as $C_0$ and $C_L$, respectively. If the membrane thickness greatly exceeds the pore radius or interfiber spacing, then near-equilibrium conditions will exist at the two boundaries, such that
When it is advantageous to switch from the local hindrance factors involved. Accordingly, when analyzing sieving, it is advanced to two products, and not on the three individual quantities. In other words, sieving coefficients depend only on those hindered severely even when \( r \).

The theoretical effects of solute charge, shape, and concentration on partitioning are discussed in section VI.

For ultrafiltration into a dead-end chamber (such as Bowman’s space), the filtrate concentration is determined by the ratio of the solute flux to the volume flux. That is, \( C_L = C/L \). With the boundary conditions now established, Equation 2 is integrated over the membrane thickness, using the requirement from conservation of mass that \( \frac{dN}{dx} \) be independent of \( x \). The result for the sieving coefficient (\( \Theta = C_L/C_0 \)) is

\[
\Theta = \frac{\Phi K_c}{1 - e^{-W} + \Phi K_c e^{-W}} = \frac{W}{1 - e^{-W} + We^{-W}}
\]

(4)

where \( Pe \), the Peclet number, is a dimensionless parameter that measures the importance of convection relative to diffusion. From the first forms of Equations 4 and 5, it is evident that the effects of membrane selectivity on sieving are contained fully in the products \( \Phi K_c \) and \( \Phi K_d \). In other words, sieving coefficients depend only on those two products, and not on the three individual quantities involved. Accordingly, when analyzing sieving, it is advantageous to switch from the local hindrance factors \( K_c \) and \( K_d \) to overall hindrance factors defined as \( W = \Phi K_c \) and \( H = \Phi K_d \). That substitution yields the second forms of Equations 4 and 5. An expression equivalent to Equation 4 was presented originally by Spiegler and Kedem (293) some 40 years ago.

The behavior of \( W \) and \( H \) for spherical solutes in cylindrical pores is shown in Figure 12. Because \( \Phi < 1 \), each overall hindrance factor is smaller than the corresponding local one; the differences are amplified as the relative molecular size increases, because \( \Phi \) becomes smaller. When measured in terms of \( H \), the rate of diffusion in a cylindrical pore is half that in free solution when \( \lambda = 0.14 \). In terms of \( W \), convective transport is halved when \( \lambda = 0.39 \). Thus solute transport in pores can be hindered severely even when \( r \) does not closely approach \( r_p \).

Equation 4 shows that the sieving coefficient depends on just two dimensionless parameters, \( W \) and \( Pe \). Of course, the diffusional hindrance factor \( H \), filtrate velocity \( v \), membrane thickness \( L \), and solute diffusivity in bulk solution \( D_v \) all influence \( Pe \), but none of those quantities affects sieving independently. The dependence of \( \Theta \) on \( Pe \) for several values of \( W \) is illustrated in Figure 13. In each case, \( \Theta \) decreases from 1 to \( W \) as \( Pe \) increases from 0 to \( \infty \); \( Pe = 5 \) is large enough to be practically infinite. Recalling that \( Pe \propto vL \), the dependence of \( \Theta \) on \( Pe \) indicates that the selectivity of an ultrafiltration membrane will be fully evident only if the product of filtrate velocity and membrane thickness is large enough to make convection dominant. Helping to amplify the effects of convection is the fact that, typically, \( W/H \approx 1 \) for porous or fibrous membranes, as discussed in connection with Figure 12. When \( Pe \) is not large, the time scale for diffusion is comparable to or less than the transit time through the membrane, thereby allowing at least partial equilibration of the filtrate with the retentate. If \( Pe \to 0 \), then \( \Theta \to 1 \) and the intrinsic selectivity of the membrane (as embodied in \( W \)) is completely masked.

The foregoing suggests that ultrafiltration will be an ineffective separation process unless \( Pe \) for each macromolecule of interest exceeds some minimum value. As indicated in Figure 13, the minimum \( Pe \) needed to achieve a desired sieving coefficient depends on \( W \). For example, the value of \( Pe \) required to make \( \Theta \) as small as 0.3 clearly increases with increasing \( W \). Expanding the exponentials in Equation 4 for small \( Pe \) shows that

\[
\Theta = \frac{W}{W + Pe(1 - W)} \quad (Pe \ll 1)
\]

(5)

This result, which is accurate to within a few percent for \( Pe < 0.1 \), indicates that ultrafiltration is effective even for
small Pe, as long as Pe greatly exceeds \( W \). More precisely, the requirement is that Pe \( \gg W/(1 - W) \). In other words, thin membranes or membranes subjected to low filtration rates must be especially selective (i.e., have very small values of \( W \)) to exhibit sieving.

The combined effects of pore radius and filtration rate on sieving curves (plots of \( \Theta \) vs. \( r_s \)) are illustrated in Figure 14. The baseline condition is a hypothetical situation in which \( r_p = 5.0 \text{ nm} \) and the product \( vL \) is such that Pe \( = 1 \) for \( r_s = 3.0 \text{ nm} \). The other predictions correspond to a selective increase in pore radius (to 6.0 nm) or a selective decrease in filtrate velocity (to one-half of the baseline \( v \)). In each case, \( \Theta \) declines from 1 to 0 as molecular size increases. Because Pe is not uniformly large, none of the curves in Figure 14 has quite the same shape as the \( W \) curve in Figure 12. A selective increase in pore radius shifts the entire sieving curve to the right, as one might expect. The effects of a selective decrease in filtration rate are more complicated, in that the sieving curve of small molecules resembles that for an increased pore radius, but the curve for large molecules is unchanged.

To understand these effects of filtration rate, one must realize that each curve corresponds to a wide range of Pe values, since Pe increases with molecular size due both to decreases in \( D_s \) and increases in \( W/H \). If the initial value of Pe is moderate, a reduction in it will increase the sieving coefficient, as shown in Figure 13. In Figure 14, the absence of any noticeable change in \( \Theta \) for the larger molecules, when \( v \) was reduced, indicates that their Pe values remained large enough to be in the plateau region of Figure 13. A practical conclusion from Figure 14 is that filtrate velocities must be monitored to avoid mistaking a flow-induced shift in a sieving curve for one caused by a change in the membrane properties.

The foregoing discussion of sieving in homogeneous membranes provides the background needed for a critical examination of a model for albumin sieving proposed recently by Smithies (292). The fundamental assumptions in that model are as follows: 1) barrier selectivity resides entirely in the GBM, 2) albumin transport across the GBM is mainly by diffusion, and 3) diffusion there proceeds independently of convection (water filtration). An implication of assumptions 2 and 3 is that variations in GFR have almost no effect on the filtered load of albumin; if GFR is increased or decreased, the albumin concentration in Bowman’s space simply would be diluted to a greater or lesser degree, with the concentration-GFR product remaining nearly the same. If assumptions 1 and 2 are correct, then the glomerular barrier behaves as a homogeneous membrane and Pe for albumin is small, making Equation 6 applicable. For \( \Theta \) to be small, as is true for albumin (Table 1), Equation 6 requires that \( W \ll \text{Pe} \), in which case it reduces to \( \Theta \sim W/\text{Pe} \). Because Pe is proportional to \( v \) and therefore (if the number of nephrons remains constant) proportional to GFR, this is consistent with the idea that filtered load will not vary with GFR if the barrier properties (i.e., \( W \)) are not affected. However, the data of Rippe et al. (251) for Ficoll sieving in rats in which GFR was manipulated show a dependence of \( \Theta \) on GFR that is far too weak to keep filtered loads constant. Similar findings were reported for the sieving of albumin and other proteins in rat kidneys (179). Thus the available data do not support the idea that the filtered load of albumin or similar-sized macromolecules is nearly independent of GFR.

Given the ample evidence that both endothelium and epithelium participate in glomerular barrier selectivity (see sects. vi and vii), assumption 1 in the Smithies model is its most obvious oversimplification. However, assumption 3 is also incorrect in general, as may be seen by returning to Equation 2. As noted earlier, steady-state conservation of mass requires that \( N \) and \( v \) be independent of \( x \). Because \( C \) decreases with increasing \( x \), the absolute value of the concentration gradient \( -dC/dx \) must increase with \( x \), leading to a \( C(x) \) profile that is concave downward. The amount of curvature in \( C(x) \) is determined by Pe. Thus flow influences diffusion by altering the shape of the concentration profile. Moreover, the contributions of convection and diffusion to \( N \) each vary with \( x \) so that, in general, it is meaningless to assert that a specific fraction of the transmembrane flux is due to one mechanism or the other. The exception is \( \text{Pe} = 0 \), where there is no convection at all, but then there is also no sieving!

**FIG. 14.** Sieving in a homogeneous membrane for three hypothetical conditions. The baseline case corresponds to a pore radius \( (r_p) \) of 5.0 nm. The other curves are those predicted for a selective increase in pore radius to 6.0 nm or a selective decrease in the filtrate velocity to one-half of the baseline level. See text for other parameter values.
C. Effects of Molecular Charge, Shape, and Concentration on Membrane Selectivity

The effects of molecular charge and shape are incompletely understood, even for cylindrical or slit pores. A major impediment to theoretical progress has been the difficulty in solving the sets of differential equations that describe the hydrodynamic forces acting on charged and/or aspherical particles in small channels. Even more formidable computational problems arise when the concentration of one or more macromolecules is large enough to make solute-solute interactions important. Given this incomplete state of knowledge, it has been common to assume that \( K_c \) and \( K_d \) are affected mainly by the Stokes-Einstein radius of the molecule and to focus on how the other factors might affect the equilibrium partition coefficient \( \Phi \). As already seen (Eq. 4), partitioning influences sieving via the overall convective hindrance factor, \( W = \Phi K_c^{-1} \); it does not affect Pe.

1. Charge effects

The simplest way to describe the effects of charge on \( \Phi \) is to model the solute of interest as a point charge with a specified valence and to assume Donnan equilibria between the membrane and adjacent solutions. In such calculations, the membrane is treated as a solution that contains a certain concentration of fixed charges, in addition to mobile ions. The fixed charges may arise from covalently attached acidic or basic groups or adsorbed ions. Structural information such as pore radius, fiber concentration, and fiber radius is neglected in Donnan models. If an external solution contains nearly impermeant ions, such as large proteins, then they are viewed as fixed charges in that solution. An excess of fixed negative charges in the membrane creates a negative intramembrane potential, which reduces \( \Phi \) for anionic macromolecules and increases it for cationic ones. There have been several applications of Donnan models to the glomerulus (53, 76, 301, 302, 313, 327, 348).

A more realistic approach is to recognize that membrane fixed charges will be localized on pore walls or on the surface of membrane fibers, rather than distributed uniformly over the membrane volume. A surface charge creates a region of opposing charge in the adjacent solution called the diffuse double layer, and it is only within that region that the electrical potential is perturbed by the surface charge. The double-layer thickness is on the order of the Debye length, which for physiological saline solutions is \( \sim 0.8 \text{ nm} \). Thus, within a porous or fibrous membrane that freely passes molecules up to 2 nm in radius, and allows some passage of macromolecules as large as 6 nm, the electrical potential is not spatially uniform, as assumed in the Donnan approach. Moreover, the charge on a multivalent macromolecular solute will be distributed in some manner over its surface or volume, rather than concentrated at a point. By computing position-dependent electrostatic potentials using the differential equations that describe diffuse double layers, \( \Phi \) has been evaluated for solid or porous spheres in cylindrical pores (291) and for solid spheres in dilute fiber matrices (136, 137).

An advantage of these more detailed calculations is that they include the interplay between the effects of molecular size and molecular charge. As the results show, the effects of size and charge on \( \Phi \) are not entirely separable. A limitation, in common with attempts to predict size selectivity, is that the actual structure of a membrane may be much more complex than the available idealizations (e.g., uniform cylindrical pores or randomly oriented fibers). The fiber model has been suggested as an alternative to the Donnan model in describing glomerular charge selectivity (52, 122, 134).

2. Shape effects

Equilibrium partition coefficients in uncharged systems have been computed for many combinations of solute shape and membrane structure. These include various shapes of rigid particles in pores of uniform cross-section (95, 155), spherical (211) or ellipsoidal (166) solutes in random fiber matrices, and freely jointed chains in pores (80) or random fiber matrices (344). The fiber matrices considered include randomly positioned and oriented fibers of a single radius (211, 344) or mixtures with multiple fiber radii (166). It is reasonable to approximate globular proteins and Ficoll as rigid particles, whereas the freely jointed chain may be a better model for dextran or other linear polymers. Such molecules do not have a fixed shape in solution and behave more as random coils.

Owing to the gel-like nature of the endothelial glyocalyx and GBM and the physiological importance of globular proteins, the partitioning of rigid particles in fiber matrices is especially pertinent. In such systems, the effects of molecular shape seem to be of little importance, unless the solutes are markedly elongated or they are random coils (see sect. iv). For example, the hydrodynamic properties of albumin resemble those of a prolate (elongated) ellipsoid with a major semiaxis of 7.0 nm and a minor semiaxis of 2.1 nm (167). Using the excluded volume theory (166), we can compare the partition coefficient of such a molecule with that of a sphere of the same Stokes-Einstein radius, 3.6 nm. In a fiber matrix with a fiber radius of 1.0 nm, \( \Phi \) for the ellipsoid is predicted to be within 1% of that for the sphere of equal diffusivity, for all fiber volume fractions from 0 and 0.2. This is consistent with partitioning (167) and sieving studies (141) in agarose gels, in which the results for albumin and certain other globular proteins
were nearly identical to those for Ficolls of equivalent Stokes-Einstein radius.

3. Concentration effects

In most applications of hindered transport theory to ultrafiltration, whether in the glomerulus or other kinds of barriers, each solute is assumed to cross the membrane independently. However, repulsive forces among like molecules, due to steric and electrostatic interactions, cause Φ to increase with increasing concentration (5, 97). For uncharged spheres, this effect is noticeable even when the solute occupies only a small percentage of the solution volume. Solute-solute interactions exist also among unlike solutes. For example, increasing the concentration of an abundant solute is predicted to increase both Φ of that solute and Φ of a second molecule present at tracer concentrations (166). This prediction has been confirmed quantitatively by partitioning measurements for BSA (abundant solute) and Ficoll (tracer) in agarose gels (167), and also helps to explain the effects of BSA on Ficoll sieving in filters constructed from isolated GBM (168). When Φ is concentration dependent, the partition coefficient at the upstream side of an ultrafiltration membrane must be distinguished from that at the downstream side (168); in Equation 4, the upstream value of Φ (or W) must be used in the numerator and the downstream value in the denominator.

Although rarely included in ultrafiltration models, concentration effects may be physiologically significant. For example, using a two-fiber model for GBM and assuming a solution protein concentration of 6 g/dl, the partition coefficients of spherical tracers with 2.0 ≤ r ≤ 5.0 nm were predicted to be 1.4–3.9 times greater than in the absence of protein, the percentage increases being greater for the larger molecules (168). The effects of pure BSA, or a 1:1 mixture of BSA and IgG, were predicted to be nearly identical. Under these conditions, the partition coefficient for BSA itself was predicted to increase 2.1-fold, relative to a very dilute BSA solution. For the glomerulus in vivo, the effects of protein concentration on plasma protein or exogenous tracer sieving will depend on the extent to which large proteins such as albumin penetrate the capillary wall. If high protein levels exist only in the capillary lumen, the effect will be limited to the partition coefficient between the lumen and endothelial glycocalyx (168). This issue is discussed further after Equation 8.

The aforementioned estimates of the concentration dependence of Φ are for uncharged solutes and membranes. Electrostatic interactions are absent in agarose gels (167) and minor in isolated GBM (28) but may be quite important in the GAG-rich glycocalyx. The combined effects of solute charge and concentration have been calculated for cylindrical pores (194), but not for fiber matrices.

D. Sieving in Multilayer Membranes

Because the glomerular filtration barrier consists of three layers in series (fenestrated endothelium with its glycocalyx, GBM, and epithelial filtration slits with their slit diaphragms), it is important to understand how sieving in a multilayer membrane differs from that in a homogeneous one. We begin quite generally. Suppose that a membrane consists of n layers arranged in series, numbered 1, 2, ..., n from the upstream to the downstream side. The convective hindrance factor and Peclet number for layer i are denoted as W_i and Pe_i, respectively. The derivation of Equation 4 can be generalized to give an expression for the sieving coefficient in layer i. Denoted as Θ_i, that sieving coefficient equals the downstream-to-upstream concentration ratio within that particular layer. The result is (68)

$$\Theta_i = \frac{W_i}{\Theta_{i+1}} \cdot \Theta_{i+2} \cdots \Theta_n (1 - e^{-Pe_i}) + W_i e^{-Pe_i}$$

(7)

where Θ_{n+1} = 1 by definition. For the most downstream layer (i = n), Equation 7 reduces to Equation 4, indicating that Θ_n is a function only of W_n and Pe_n. In other words, the sieving coefficient in the last layer is independent of the selectivity or flow conditions in the preceding layers. However, as indicated by the product of sieving coefficients in the denominator of Equation 7, all of the other sieving coefficients are coupled to some extent, each being affected by events downstream. This coupling is most complete for the first layer (i = 1), in that Θ_i is influenced by all of the other sieving coefficients. The fact that the sieving coefficient in a given layer influences all of the upstream ones, but none of the downstream ones, indicates that the ordering of the layers is important. The effects of ordering in a two-layer membrane will be examined shortly.

The interdependence of the layer sieving coefficients stems from two physical constraints. One is that the water and solute fluxes across each layer must be the same. Once a steady state has been reached, there can be no time-dependent accumulation or depletion of water or solute at any boundary between layers. The other constraint is that the concentration at the downstream side of layer i is linked to that at the upstream side of layer i + 1. The solute concentration profile in each layer must adjust accordingly, and Equation 7 is a consequence of those simultaneous adjustments.

For a multilayer barrier and dilute retentate, the overall sieving coefficient is calculated as
\[ \Theta = \Theta_1 \Theta_2 \ldots \Theta_n \] 

(8)

Of course, \( \Theta \) measures how well the barrier functions as a whole. If the retentate is not dilute, one or more additional, multiplicative factors will appear in Equation 8 (168), depending on the extent to which abundant solutes penetrate the barrier. For example, if the major plasma proteins are excluded from the endothelial glyocalyx, such that high protein concentrations occur only in the lumen, the additional factor equals the relative increase in \( \Phi \) for the glyocalyx that is due to the plasma proteins (168). In other words, if the luminal protein concentration is sufficient to double \( \Phi \) in the glyocalyx, Equation 8 will contain an additional factor of 2. For simplicity, we will proceed as if all solutions are dilute.

Examining the behavior of a two-layer membrane provides insight into how layers of the glomerular barrier may interact. For \( n = 2 \), Equation 7 applied to the upstream layer becomes

\[ \Theta_1 = \frac{W_1}{\Theta_2 (1 - e^{-Pe_1}) + W_1 e^{-Pe_1}} \] 

(9)

The influence of the downstream layer on sieving in the upstream one is most striking for \( Pe_1 \gg 1 \), in which case Equation 9 reduces to \( \Theta_1 = W_1/\Theta_2 \). If the upstream layer is less selective than the downstream one, in the sense that \( W_1 > \Theta_2 \), then \( \Theta_1 > 1 \). This indicates that an internal concentration polarization is created, such that the solute concentration in layer 1 increases in the direction of flow. Concentration polarization in the upstream layer is avoided if \( W_1 < \Theta_2 \). However, for large \( Pe_1 \), the overall sieving coefficient for the two layers is always \( \Theta = \Theta_1 \Theta_2 = W_1 \). It is remarkable that, under these circumstances, \( \Theta \) is independent of the properties or flow conditions in the downstream layer. For \( Pe_1 \ll 1 \), Equation 9 reduces to

\[ \Theta_1 = \frac{W_1}{W_1 + Pe_1 (\Theta_2 - W_1)} \] 

(10)

which is the two-layer analog of Equation 6. In this case, both \( \Theta_1 \) and \( \Theta \) depend on the properties of each membrane layer and are also affected (through \( Pe_1 \)) by the filtration rate of water.

Suppose that layer 1 is the GBM and layer 2 is the epithelium. For a molecule the size of albumin, it has been estimated that \( Pe_1 = 0.14 \) (73), based on the sieving and diffusion properties of isolated rat GBM and the dimensions and flow rates for rats in vivo. With the use of \( Pe_1 = 0.14 \), together with the finding in isolated rat GBM that \( W_1 = 0.08 \) for a Ficoll molecule the size of albumin (73), either Equation 7 or Equation 10 indicates that \( \Theta_1 \sim 1 \) for any small value of \( \Theta_2 \). Thus it was concluded that if the slit diaphragm is a significant contributor to albumin sieving, the GBM is not (73).

The conclusion that the GBM does not influence albumin sieving, just described, is at odds with the finding that selective alterations in mouse GBM can lead to proteinuria (132). If in reality the GBM contributes significantly to albumin sieving in normal animals, \( W_1 \) would need to be orders of magnitude smaller than that measured in vivo. (For example, to yield \( \Theta_1 = 0.1 \) at \( Pe_1 = 0.14 \), then \( W_1 = 0.0014 \) for \( \Theta_2 = 0.1 \) and \( W_1 = 0.00014 \) for \( \Theta_2 = 0.01 \).) It is possible that loss of macromolecular constituents during GBM isolation led to a “looser” material with an elevated \( W \). However, theories of flow through fibrous media indicate that loss of, say, GAGs, also would greatly elevate the hydraulic permeability (27). What is most difficult to reconcile is that the hydraulic permeability in isolated GBM (measured simultaneously with Ficoll sieving) was low enough to account for nearly all of the known resistance to water filtration in vivo (73). That is, a much “tighter” GBM in vivo would likely give unrealistically low values of single-nephron GFR.

In a multilayer barrier, physical interactions among the layers also influence the dependence of the overall sieving coefficient on the water filtration rate. As discussed already, \( \Theta \) for a homogeneous membrane invariably declines as the filtration rate increases, until at high \( Pe \) it reaches a minimum value equal to \( 1 \). That is not necessarily the case for a multilayer membrane, as will be illustrated next.

For simplicity, we again consider a barrier with just two layers. In general, the value of the Peclet number in a given layer may be small, moderate, or large, suggesting that there are nine combinations to consider when exploring how two-layer membranes behave. However, small values of \( Pe_1 \) or \( Pe_2 \) are less interesting, because a layer with a small Peclet number will tend to be “invisible” (unless \( W_i < Pe_i \)). Of the four Peclet number combinations that remain, only three give different results for the overall sieving coefficient, as discussed recently (68) and explained in the three cases that follow. We will use “\( Pe \sim 1 \)” to indicate a moderate \( Pe \) and “\( Pe \gg 1 \)” to indicate a large \( Pe \). More precisely, \( Pe \sim 1 \) means that neither \( \exp(-Pe) \) nor \( 1 - \exp(-Pe) \) is negligible compared with unity. If errors of \(<20\%\) are acceptable, that will be true for \( Pe \) values ranging from \(~0.2\) to 2.

1. Case 1

If \( Pe_1 \gg 1 \), then it follows from Equations 7 and 8 that \( \Theta_1 = W_1/\Theta_2 \) and

\[ \Theta = W_1 \] 

(11)

As already discussed, the overall sieving coefficient is independent of the filtration velocity in this case and is
determined entirely by the selectivity of the upstream layer. The downstream layer has no effect here, even if Pe₂ is not small. To obtain this result, Pe₁ must be large enough to make W₁ exp(−Pe₁) negligible compared with Θ₂.

2. Case 2

If Pe₁ ≈ 1 and Pe₂ ≫ 1, then Θ₂ = W₂ and

\[ \Theta = \frac{W₁ W₂}{W₂ + (W₁ - W₂) e^{-Pe₁}} \]  \hspace{1cm} (12)

Here the sieving coefficient is flow dependent and is influenced by the selectivity of both layers. Since Pe₁ is proportional to the filtration rate of water, Θ will increase with the filtration rate if W₁ > W₂ and decrease with filtration rate if W₁ < W₂.

3. Case 3

If Pe₁ ≈ 1 and Pe₂ ≈ 1, there is no algebraic simplification, and the resulting expression for Θ is too unwieldy to offer much insight. However, if Pe₁ = Pe₂ = Pe, the result is

\[ \Theta = \frac{W₁ W₂}{W₂ + (W₁ - W₂) e^{-Pe₁} - W₁ (1 - W₂) e^{-2Pe₁}} \]  \hspace{1cm} (13)

which is valid for any Pe. As in case 2, the sieving coefficient is flow dependent and influenced by the properties of both layers. If Pe → 0 in Equation 13, then Θ → 1, as seen before. If Pe ≫ 1, Equation 13 reduces to Equation 11. A comparison of Equations 12 and 13 reveals an additional term in the denominator of the latter, which is negative and which therefore tends to increase Θ at any intermediate value of Pe. Thus the intrinsic selectivity of the two layers is used less efficiently in the conditions assumed for Equation 13 than in those for Equation 12.

It is informative to synthesize a two-layer membrane mathematically, starting with one layer and then adding a second with the same or different properties. Keeping in mind that the sieving coefficients for large molecules (e.g., albumin) should be as small as possible, we can inquire whether the overall barrier function is improved or worsened by adding a second layer. The results of such calculations are given in Figure 15, which shows the overall sieving coefficient as a function of the Peclet number in layer 1. The curve for the single membrane was generated using W = 0.01 in Equation 4 and is identical to the bottom curve in Figure 13. If we now add a second layer that has the same properties as the first, creating a “double membrane,” there tends to be a downward shift in Θ for any given value of Pe₁. For the arbitrary parameter values that we chose for Figure 15, the maximum effect (a 45% reduction in Θ) occurs at a Pe value of ~0.1. Because the two layers have the same intrinsic selectivity, the double membrane is the same as a homogeneous one with twice the original thickness. Recalling that the Peclet number is proportional to membrane thickness, it is apparent that for the double membrane the Peclet number to be used in Equation 4 must increase from Pe₁ to 2Pe₁. The reduction in Θ occurs only at small-to-moderate Pe₁, because when Pe₁ is large, there is no benefit in doubling it. That is, for large Pe₁ the sieving coefficient is already at its minimum value, Θ = W. The assertion that adding an identical second layer merely doubles the apparent value of Pe can be verified by setting W₁ = W₂ = W in Equation 13.

If the additional layer is an order of magnitude less selective, the results are very sensitive to its position. If it is placed downstream, such that W₁ = 0.01, W₂ = 0.1, and Pe₂ = Pe₁, barrier function is improved. However, the values of Θ obtained from Equation 13 (not shown) are at most 7% lower than those for the single-layer membrane, changes that are too small to be evident on the log scale in Figure 15. If the additional layer is placed upstream, such that W₁ = 0.1, W₂ = 0.01, and Pe₂ = Pe₁, barrier function is worsened. In this case, Equation 13 predicts marked elevations in Θ at both moderate and large values of Pe₁, relative to the single-layer membrane. For Pe₁ > 5 (somewhat beyond the range of Fig. 15), the overall sieving coefficient closely approaches that of the upstream layer, consistent with Equation 11.

The functional degradation seen when the less selective layer is placed upstream reflects internal concentration polarization within the first membrane layer. In gen-

FIG. 15. Sieving in single-layer and two-layer membranes. The overall sieving coefficient (Θ) is plotted as a function of the Peclet number in layer 1 (Pe₁). Curves are shown for a single-layer membrane (W₁ = 0.01); a membrane in which a second, identical layer has been added upstream or downstream from the first (W₁ = W₂ = 0.01); and a membrane with a second, less selective layer added upstream from the first (W₁ = 0.1, W₂ = 0.01). In the two-layer membranes, it was assumed that Pe₁ = Pe₂.
In the nephrotic syndrome, more than 300 mg protein per day (or 200 mg/l).

A. What is Proteinuria?

Proteinuria is said to be present when the urine contains more than 300 mg protein per day (or 200 mg/l).

B. Where do Proteins in the Urine Come From?

Proteins in the final urine have different origins. First, proteins cross the glomerular barrier, and tubular cell reabsorption modifies their final concentration. Second, there is tubular secretion of proteins from the blood. Third, proteins may be synthesized by the cells themselves and released into the urine. Fourth, the proteins may be added to the urine at a later stage (e.g., by excretion from the prostate gland in men). Let us examine these alternatives in more detail.

Patients with glomerulonephritis have proteinuria, hematuria, elevated blood pressure, and morphological alterations in glomerular structures. It has therefore been natural to assume that the proteinuria is caused by defects in the glomerular barrier. Recently, this view has been questioned and an alternative hypothesis has been proposed, the so-called albumin retrieval hypothesis (84). The hypothesis has attracted certain interest, and it is therefore discussed below, together with the overwhelming evidence against it.

C. The Albumin Retrieval Hypothesis

According to the albumin-retrieval hypothesis, the proteinuria that occurs with diabetic nephropathy (226, 229) or other renal disorders (265) reflects defects in the tubular system with intact glomerular permeability (265, 266). The morphological changes in the glomerulus are therefore considered coincidental and of no pathophysiological significance. The hypothesis rests on four assumptions: 1) that albumin is retrieved from the tubule fluid in intact form (84); 2) that the glomerular sieving coefficient for albumin is close to 0.08 (228), which is two to three orders of magnitude higher than other estimates (see Fig. 10); 3) that the uptake of albumin is mediated by the megalin-cubulin complex (264); and 4) that albumin is degraded and fragmented in the urine (84). None of these assumptions is valid.

First, the question of whether there is any tubular uptake of intact albumin is not a new one. In 1966, Maunsbach studied the tubular uptake of autologous rat albumin (185) and concluded that such uptake must be extremely small or nonexistent (184). Others have confirmed and extended these findings. Measurements of the kinetics of albumin uptake in proximal tubules (231) led to the conclusion that albumin is taken up by the tubular cells at the luminal side, and its amino acids are released on the other side to blood (231). The molecular mechanism is the megalin-cubulin complex (21, 22).

Second, a glomerular sieving coefficient for albumin close to 0.08 is not compatible with life and must be due to flaws in the experimental technique. A simple example illustrates the magnitude of the problem. Thus, for a person with a glomerular filtration rate of 180 liters/day, a serum concentration of albumin close to 40 g/l, and a sieving coefficient for albumin of 0.08, the daily losses of albumin would be more than half a kilogram (180 × 40 × 0.08 = 576 g). With the assumption that a reuptake of intact albumin exists (despite compelling evidence against it, see above), such amounts are higher than theoretically achievable using detailed models of tubular uptake (169).

Third, it has been suggested that the retrieval of intact albumin is mediated by the megalin-cubulin complex (264). Indeed, megalin and cubulin are strongly linked to the uptake of albumin and several other impor-
tant solutes in many species, including humans (49, 337). It is also known that the turnover of the megalin-cubulin complex is controlled by a chloride channel, CIC-5 (50, 124), which opens new therapeutic avenues (157). However, the megalin-cubulin complex cannot be responsible for the reuptake of intact albumin, since these complexes are internalized into lysosomes, and the albumin is degraded (49).

Fourth, one research group concluded that albumin fragments are present in urine and require special techniques to be detected (54, 56, 84, 100, 120, 221, 265, 295). That conclusion is based on the assumption that their method, which uses both the Biuret reagent and an HPLC technique, is superior to the methods used by others. The Biuret reagent is assumed to be sensitive and specific for protein detection. Norden et al. (209) tested this assumption using state-of-the-art proteomics matrix-assisted laser desorption/ionization time of flight mass spectrometry and Nano liquid chromatography electrospray tandem mass spectrometry, to select 14 nonpeptide components from urine, mix them, and test the samples using the Biuret reagent.

Although there were no proteins present in these samples, the Biuret reagent gave a protein value of 0.4 g/l, and similar errors were found with the HPLC technique (209). Moreover, a proteomics analysis of the fate of albumin in patients with Fanconi syndrome and in normal controls showed no evidence of significant fragmentation of albumin in the urine (209). As mentioned in section iv, patients with Fanconi syndrome excrete large quantities of amino acids and proteins due to a defect in their tubular reabsorption process. These findings provide additional support for our current understanding of the megalin-cubulin complex, in which albumin is internalized by lysosomes, degraded, and released to the blood in the form of amino acids (49, 207). Considering the paucity of experimental support, it is surprising that the albumin retrieval hypothesis has survived so many years.

D. Human Diseases and Animal Models

Proteinuria is a hallmark of renal disease, and patients may suffer from glomerulonephritis, diabetic nephropathy, and numerous other conditions. Among the conditions that cause nephrotic syndrome (with heavy proteinuria, edema, low serum albumin concentrations, and hyperlipidemia), minimal change nephrosis is the most common disorder in children, while membranous glomerulonephritis is predominant in elderly patients. Diabetes mellitus affects 170 million people worldwide, and the number is expected to double over the next 25 years (102). This makes diabetic nephropathy the most common cause of proteinuria, and occasionally the amounts excreted reach nephrotic levels. Some patients suffer from focal segmental glomerulosclerosis, and others have primary or secondary forms of membranoproliferative glomerulonephritis. In all these cases, the diagnosis is obtained from morphological analysis of membranoproliferative glomerulonephritis. In the future, quantitative PCR will be used to assess the expression profiles of different proteins (325).

Today we know the molecular defect underlying many genetic disorders (321). Patients with nephrotic syndrome of the Finnish type have a defect in the nephrin gene, NPHS1 (155). Children with steroid-resistant nephrotic syndrome are likely to have a problem with the podocin gene, NPHS2 (29). LMX1B, a gene that is mutated in patients with nail-patella syndrome, is required for podocyte differentiation (192). Indeed, several proteins are known to be important for an intact glomerular barrier (see Refs. 83, 90, 103, 14, 192, 237, 320, 321, 326, 345). However, many renal disorders do not seem to be explained by genetic factors, and the underlying mechanisms remain unknown.

Because of our rudimentary understanding of renal pathophysiology, most treatments are nonspecific, cytotoxic, and anti-inflammatory, with potentially harmful side effects. As a result, many patients with glomerulonephritis are given conservative treatments such as diuretics and antihypertensive medicine alone. Preeclampsia is of particular interest because the disease involves endothelial dysfunction (147, 186). Women with preeclampsia have glomerular endotheliosis with swelling and vacuolization of the cells (187). Podocytes are not affected and the GBM is intact, suggesting that the proteinuria is due solely to endothelial damage. Moreover, VEGF is involved in the process (86, 186). Proteinuria also occurs in Fanconi syndrome due to defective tubular reabsorption of filtered protein (207–209) (see above).

Proteinuria also occurs after ischemia-reperfusion, which is evident from studies on transplanted kidneys (9, 294). A longer period of ischemia (60 min) causes nonselective damage to the glomerular barrier (252). If, however, the ischemia is shorter (15–20 min), the damage following reperfusion mainly affects glomerular charge selectivity (6, 252). These changes occur with no apparent damage to the podocytes, the GBM, or the endothelial cells, suggesting more subtle effects of the endothelial cell surface layer (6). In other organs, similar short ischemic periods have been shown to selectively damage the endothelial glycocalyx (198, 236, 262). Also, perfused kidneys have been reported to lose sulfated proteoglycans and GAGs after ischemia (333), supporting the idea that ischemia reperfusion causes proteinuria through damage of the endothelial surface layer.

Several animal models have been used to mimic kidney disease in humans. Most studies of nephrotic syndrome have been done using nephrosis-inducing agents such as puromycin aminonucleoside (122, 123, 216, 220, 225) and adriamycin (18, 19, 161). More recently, studies
in mouse knockout models have provided more information on the specific actions of individual proteins such as laminin (350) and lamb2 (132), suggesting a major role for the GBM in proteinuric conditions. Furthermore, induction of B7-1 in podocytes gives a nephrotic syndrome in mice (241). Mice lacking the podocyte slit membrane CD2-associated protein develop congenital nephrotic syndrome (287). There are mice that develop a disease resembling focal segmental glomerulosclerosis (125, 126) and many other models (127). Most investigators try to develop tissue-specific inducible genetic models to suppress or overexpress a certain protein. More sophisticated techniques can be used to study glomerular size and charge selectivity in these mice (133–135), rather than simply determining if proteinuria is present. The variety of animal models and the continuing discovery of genes mutated in patients with kidney disease give us a firm platform for elucidating the mechanisms of proteinuria.

VIII. SUMMARY

In life, we search for simple answers to complex questions. With respect to the glomerular barrier, scientists have presented data suggesting that the GBM, the podocyte, or the endothelium is the one and only important component. In this review, we argue for an integrative view of the glomerular barrier and of the mechanisms behind proteinuria. From the theoretical analysis presented above, it is evident that defects in any of the three glomerular layers may result in proteinuria. Indeed, a quest is under way for a more global analysis of glomerular proteins affected by disease (305).

A. What is the Relevance of the Individual Components of the Glomerular Barrier?

The slit membrane of the podocytes is the most complex structure in the glomerular barrier and definitely of great importance for glomerular selectivity. As discussed already, several diseases in humans and animals are caused by gene polymorphisms that affect proteins involved in the slit membrane. Whether the podocyte layer contributes to both charge and size selectivity is a matter of debate. There simply are no data to support opinions on this matter. The GBM, on the other hand, shows some size selectivity but little charge selectivity, at least when studied in isolated form using current protocols (28). Finally, the capillary endothelium is gaining more and more attention (323). The high frequency of large fenestrae has led many investigators to assume that the endothelium does not contribute to the barrier, apart from keeping blood cells from the GBM. However, data from other capillary beds with fenestrated endothelia (307) suggest these structures are as selective as continuous capillaries, such as those in skeletal muscle (250). Indeed, the sieving coefficient for albumin in peripheral capillaries is <0.1, due to the properties of the endothelial surface coat or to the fiber matrix nature of underlying basement membrane-like structures (55, 259). In Figure 5, we have illustrated the endothelial cell surface layer with some key components. The endothelial surface coat has been studied quite extensively in other organs (204, 328, 329, 332, 338), but the visualization is mainly indirect, and disease models are lacking (323). Nevertheless, it seems safe to conclude that glomerular capillary endothelium is at least as selective as that in intestine and in salivary and pancreatic glands. If so, the concentration of albumin in the fluid entering the GBM is likely to be 10% or less of that in plasma.

The highly differentiated and specialized podocyte is of utmost importance for an intact barrier. Several genetic defects in the podocyte affect its structure (321, 322) and cause diseases with proteinuria. In addition, through the production of growth factors such as VEGF, the podocyte affects the GBM and the endothelium indirectly (66). Regardless of where the defect is, the result is proteinuria. Thus the glomerular barrier with its specialized components truly acts as an integrative and delicate structure.

Over the next decade, a deeper understanding of the barrier will lead to insight into the pathogenic mechanisms of kidney diseases and provide specific therapies. The glomerular barrier is highly size and charge selective in a manner that can be predicted using modern transport theories (see Fig. 11). In particular, it markedly restricts the passage of large anionic proteins such as albumin. If these capillaries were as permeable as peripheral capillaries in skeletal muscle, a normal man would lose close to a kilogram of albumin per day in the urine (116). Fortunately, we lose <1% of that amount into the primary urine and <30 mg/day of albumin in the final urine. The components of the glomerulus that create such a magnificent barrier are the podocytes, the GBM, and the fenestrated endothelial cells with their endothelial surface layer of GAGs.

Most known pathologies have been found in podocytes, in particular in proteins of the podocyte slit membrane (319, 323). The GBM constitutes most of the glomerular hydraulic resistance (65), while being highly permeable to solutes. Thus the GBM does not seem to be particularly charge or size selective (28). Still, some GBM lesions, such as mutations of the laminin β2-chain (350), produce proteinuria even in the absence of detectable changes in the cellular components (132). This finding is not readily compatible with functional studies of the GBM (68), unless laminin is present in the endothelial cell surface layer as well. The endothelium has hitherto been neglected (68, 70, 116). However, recent data on preeclampsia (147, 171, 172, 187) show that this endothelial disease may affect glomerular capillaries and cause pro-

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teinuria through the actions of VEGF (147). Indeed, glomerular lesions similar to those in human preeclampsia were found in a mouse model with altered expression of VEGF-A in the podocytes (85). Studies on kidneys subjected to mild ischemia support the notion that proteinuria may be caused by defects in the endothelial surface layer (6).

B. What Do We Need to Know More About?

Despite recent advances, the mechanisms underlying acquired glomerular disorders are poorly understood. As a result, the therapeutic arsenal is limited and blunt. Regarding the properties of the glomerular barrier, more biological data are needed on the transglomerular passage of proteins that differ in size, charge, and shape. Different technical approaches should be adopted, since each method has advantages and drawbacks. In particular, the impact of molecular configuration must be studied in detail. Only limited amounts of biological data have been obtained under conditions of controlled tubular cell activity. We need to learn more about the individual components of the barrier. The podocyte is the most complex and fascinating part of the glomerular membrane, and despite a decade of intense research, much remains to be learned about this structure. For example, it will be important to understand in greater detail the communication between podocytes and the endothelium. It will also be important to elucidate the endothelial and epithelial production of proteoglycans and GAGs. What substances are produced by these cells and at what rates? What is the composition of the endothelial cell surface layer as shown in Figure 5, and what is the turnover rate of this layer? What is the role of the mesangial cell? We need to know how various agents affect glomerular permeability during health and disease. Finally, we must use the information at hand to develop better therapies for the many patients with kidney disease.

C. Concluding Remarks

We have presented an integrative view of the glomerular barrier, according to which each component of the barrier must maintain its integrity to preserve normal function. The GBM appears to be less selective than the cellular components. The statement that podocyte damage leads to proteinuria is still accurate; it is, however, equally true that damage to the endothelium causes proteinuria. In the final section of this review, we have highlighted important questions that need to be addressed for a better understanding of one of nature’s great wonders, the glomerular barrier.

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