Structural determinants of glomerular permeability

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Deen, William M., Matthew J. Lazzara, and Bryan D. Myers. Structural determinants of glomerular permeability. Am J Physiol Renal Physiol 281: F579–F596, 2001.—Recent progress in relating the functional properties of the glomerular capillary wall to its unique structure is reviewed. The fenestrated endothelium, glomerular basement membrane (GBM), and epithelial filtration slits form a series arrangement in which the flow diverges as it enters the GBM from the fenestrae and converges again at the filtration slits. A hydrodynamic model that combines morphometric findings with water flow data in isolated GBM has predicted overall hydraulic permeabilities that are consistent with measurements in vivo. The resistance of the GBM to water flow, which accounts for roughly half that of the capillary wall, is strongly dependent on the extent to which the GBM surfaces are blocked by cells. The spatial frequency of filtration slits is predicted to be a very important determinant of the overall hydraulic permeability, in keeping with observations in several glomerular diseases in humans. Whereas the hydraulic resistances of the cell layers and GBM are additive, the overall sieving coefficient for a macromolecule (its concentration in Bowman's space divided by that in plasma) is the product of the sieving coefficients for the individual layers. Models for macromolecule filtration reveal that the individual sieving coefficients are influenced by one another and by the filtrate velocity, requiring great care in extrapolating in vitro observations to the living animal. The size selectivity of the glomerular capillary has been shown to be determined largely by the cellular layers, rather than the GBM. Controversial findings concerning glomerular charge selectivity are reviewed, and it is concluded that there is good evidence for a role of charge in restricting the transmural movement of albumin. Also discussed is an effect of albumin that has received little attention, namely, its tendency to increase the sieving coefficients of test macromolecules via steric interactions. Among the unresolved issues are the specific contributions of the endothelial glycocalyx and epithelial slit diaphragm to the overall hydraulic resistance and macromolecule selectivity and the nanostructural basis for the observed permeability properties of the GBM.

Darcy permeability; sieving coefficient; Ficoll; equilibrium partition coefficient

THE CONCEPT OF THE GLOMERULUS as a highly refined ultrafiltration device, capable of filtering large volumes of plasma while efficiently retaining proteins within the circulation, has long been one of the cornerstones of renal physiology. Although that basic view of glomerular function is due to earlier generations of researchers, efforts to achieve a quantitative understanding of glomerular filtration received a distinct stimulus some 30 years ago. Beginning about 1970, new animal models (e.g., the Munich-Wistar rat) and advances in micropuncture pressure measurement techniques per-
mitted a much more direct examination of glomerular forces and flows in mammals than had been possible previously. Those and other developments have stimulated a large number of investigations into the dynamics of water filtration and the selective retention of macromolecules by the glomerulus in health and disease. A comprehensive review is available of results published through about 1990 (66).

Among the more recent lines of research are efforts begun in the early 1990s to relate the functional properties of the glomerular capillary wall to its unique structural features, on the cellular and even macromolecular level. This represents a significant departure from earlier analyses of glomerular barrier function by us and others, which mainly sought to express the available micropuncture and clearance measurements in terms of hydraulic permeabilities and effective pore sizes. In other words, in the 1970s and 1980s the glomerular capillary wall was regarded largely as a black box with certain measurable properties, whereas more recent biophysical analyses have sought to explain its permeability properties in terms of specific structures. This has been done by combining morphometric results, in vitro data using isolated glomeruli, and detailed hydrodynamic models of the capillary wall. Such work is the focus of this review.

As background, we begin with a brief overview of the structure and composition of the glomerular capillary wall. The permeability properties will be affected by features spanning a wide range of length scales, from the dimensions of cells to the dimensions of the macromolecules that form the basement membrane and junctional complexes (slit diaphragms). Those in the ~100- to 1,000-nm and ~0.1- to 10-nm ranges are conveniently labeled as “microstructural” and “nanostructural,” respectively. Following the structural description is a section on water permeability, in which the main elements of the structure-based hydrodynamic models are discussed and their predictions compared with experimental findings in vivo. Emphasized there are insights into the reduced filtration capacity for water in several forms of glomerular disease. The last section concerns the selectivity of the glomerular barrier to macromolecules. Efforts to understand glomerular size selectivity in terms of structural models are reviewed, and various unresolved issues are discussed. One of the most controversial issues is the extent to which charge selectivity is important for glomerular barrier function. Also included is a discussion of recent findings concerning the effects of serum albumin on the sieving of macromolecular tracers.

**STRUCTURE AND COMPOSITION**

**Microstructural Idealizations**

We focus here on structural representations that have been used in modeling glomerular permeability; a much more comprehensive discussion of glomerular anatomy is available elsewhere (55). The glomerular capillary wall is unusual in having three layers: a fenestrated endothelium, the glomerular basement membrane (GBM), and the foot processes of glomerular epithelial cells. Between the epithelial foot processes are “filtration slits” bridged by slit diaphragms. Because of the low water permeability of most cell membranes, it is generally accepted that glomerular filtrate follows an extracellular path: through the fenestrae, across the GBM, and through the slits (passing through the slit diaphragms). To describe this flow, Drummond and Deen (31) proposed that the glomerular capillary wall be viewed as an assembly consisting of many repeating subunits. The basic structural subunit, as shown in Fig. 1, consisted of a single filtration slit, an associated area of GBM, and several fenestrae. The key geometric quantities in this model are the width of the structural unit (W), the thickness of the GBM (L), the width of the filtration slit (w), the dimensions of a fenestra, and the number of fenestrae per filtration slit. Representative values gleaned from various morphometric studies in rats (1, 39, 57, 62, 87, 90, 92, 98, 107) are summarized in Table 1. Typical dimensions for rats are W = 360 nm, L = 200 nm, and w = 39 nm. The extent to which the GBM surfaces are blocked by cells is described by the fraction of the surface area occupied by slits (εs = w/W = 0.11) and the fraction of the area occupied by fenestral openings (εf = 0.20). As depicted in Fig. 1, the fenestrae have been reported to have an hourglass shape (62). The value of εf is based on the minimum cross-sectional area. Other information needed to model the fenestrae is discussed in Drummond and Deen (31).

Data for healthy humans suggest a slit width similar to that in rats, w = 43 nm (37) but a significantly larger subunit width and GBM thickness, W = 500 nm and L = 400 nm, respectively (58, 97). A morphometric index used to describe slit spacing is the filtration slit frequency (FSF), which is related to the subunit width by W = (2π)/FSP; the factor 2π accounts for the random angle of sectioning (33).

![Fig. 1. Idealized structural unit of the glomerular capillary wall, corresponding to one filtration slit. The figure is modified from Ref. 35. W, width of the structural unit; L, the thickness of the glomerular basement membrane (GBM); w, the width of the filtration slit; x and z, coordinates.](http://ajprenal.physiology.org/)

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Table 1. Microstructural parameters representative of normal rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width of structural unit, ( W_0 ), nm</td>
<td>360</td>
</tr>
<tr>
<td>Thickness of GBM, ( L_0 ), nm</td>
<td>300</td>
</tr>
<tr>
<td>Width of filtration slit, ( w_0 ), nm</td>
<td>19</td>
</tr>
<tr>
<td>Fractional area of fenestrae, ( \epsilon_f )</td>
<td>0.20</td>
</tr>
<tr>
<td>Fractional area of filtration slits, ( \epsilon_s )</td>
<td>0.20</td>
</tr>
<tr>
<td>Number of fenestral openings per slit, ( n_f )</td>
<td>3</td>
</tr>
</tbody>
</table>

GBM, glomerular basement membrane.

**Slit Diaphragm**

Among the key nanostructural dimensions are those that describe the openings in the slit diaphragm. Figure 2A shows an enlarged view of the slit diaphragm oriented as in Fig. 1. The most frequently cited configuration for the slit diaphragm is that of Rodewald and Karnovsky (87), who described a structure consisting of a central filament oriented parallel to the podocyte membranes and regularly spaced bridge fibers, alternating from side to side, that connect the central filament to the membranes. This arrangement, which we term the "zipper" structure, is depicted in Fig. 2B. The reported dimensions of the openings were \( 40 \times 140 \) Å. These dimensions are problematic in that they imply a much more size-selective barrier than that shown by functional measurements, as will be discussed. A simpler structure, motivated by the observations of Hora et al. (45), is shown in Fig. 2C. This "ladder" structure remains quite tentative, and specific dimensions for it are not available from electron microscopy.

Recent efforts to elucidate the structure of the slit diaphragm have centered on its component molecules, particularly the newly identified protein nephrin. Nephrin has a molecular mass of \( \sim 150 \) kDa and has been shown to be expressed exclusively by glomerular podocytes in the slit diaphragm region (44, 89). Lack of proper expression of the nephrin gene has been shown by Tryggvason and co-workers (63, 102) to be linked to the congenital nephrotic syndrome of the Finnish type, a glomerular disorder that results in severe proteinuria and that is associated with normal GBM and the loss of foot processes and slit diaphragms. Genetic analysis of the coding region of the nephrin gene has demonstrated that it is a single-pass, membrane-spanning protein with eight Ig motifs and a type III fibronectin domain (102). It has been hypothesized that nephrin molecules extending out from adjacent podocytes might interact in a homophilic manner to form the zipper structure (102). Such proposals remain speculative, as the interaction of nephrin with other protein components of the slit diaphragm is not yet known. It has been demonstrated that cultured podocytes form linking structures that are similar to filtration slits in vivo and that these intercellular linking structures contain the proteins zonula occludens-1, P-cadherin, and \( \alpha_\perp \), \( \beta_\perp \), and \( \gamma \)-catenin (82).

**GBM**

The GBM is a gel-like material that is 90–93% water by volume (21, 85). Structural integrity is conferred by a heteropolymeric network of type IV collagen, laminin, fibronectin, entactin, and heparan sulfate proteoglycan (59, 66). Collagen IV, a triple helical polypeptide, is thought to form an interconnected network of fibers within the GBM, to which other matrix components are attached. Laminin, an asymmetrical four-armed structure, is thought to play an important role in the structural integrity of the GBM and in its interactions with the cellular layers of the glomerular capillary wall. The sulfated glycoprotein entactin, or nidogen, binds to collagen IV, heparan sulfate proteoglycan, and laminin and thus may play an important role in linking GBM components to one another. Similarly, fibronectin, a 500-kDa glycoprotein, binds to laminin, collagen IV, and heparan sulfate proteoglycan, suggesting that it too may have a role in linking GBM constituents together. Heparan sulfate proteoglycan has been shown to comprise \( \sim 1\% \) of the dry weight of the GBM (54). The predominant GBM proteoglycan is made up of a 400-kDa core protein called perlecan and four to five heparan sulfate chains bound to one end of the core protein (103). These anionic heparan sulfate chains are made of repeating disaccharide units of glucosamine and glucuronic acid (55).

**Endothelial Glycocalyx**

The glycocalyx that covers the luminal surface of the endothelial cells and fills the fenestrae may also be an important determinant of glomerular permeability. This layer is thought to be composed principally of sulfated proteoglycans (95) and glycoproteins (94). Recent electron microscopy studies (88) demonstrated a 300-nm-thick filamentous surface coating that appeared to be present over both fenestral and interfenestral surfaces. The thicknesses of endothelial surface coatings reported by Rostgaard and Qvortrup (88) exceed those previously observed by other authors (65, 93) by a factor of three to five. This difference was attributed to a novel method of tissue fixation, combined with a treatment that enhanced micrograph contrast.
FILTRATION OF WATER

Structure-Based Model

The structural unit depicted in Fig. 1 was used by Drummond and Deen (31) to formulate a hydrodynamic model for the filtration of water across the glomerular capillary wall. The objective of the model was to predict values of the effective hydraulic permeability \( k \). Because the three layers of the capillary wall act as resistances in series, the overall hydraulic permeability is related to those of the individual layers by

\[
\frac{1}{k} = \frac{1}{k_{en}} + \frac{1}{k_{bm}} + \frac{1}{k_{ep}}
\]

(1)

where \( k_{en}, k_{bm}, \) and \( k_{ep} \) are the hydraulic permeabilities of the endothelium, GBM, and epithelium, respectively. Thus the problem is reduced to that of analyzing each layer in turn and then adding the results, as shown in Eq. 1. The results for the cellular layers will be reviewed first, followed by those for the GBM.

Finite-element solutions of Stokes’ equation (the low-Reynolds-number form of the Navier-Stokes equation) were used to characterize flow in the epithelial filtration slits (30). The results indicated that the slit diaphragm is the dominant resistance to water flow between the foot processes, implying that the slit length is not an important parameter for water filtration. With the use of the zipper structure, with all dimensions as given in Rodewald and Karnovsky (87), the permeability of the slit diaphragm (in SI units) was estimated as \( k_s = 7.9 \times 10^{-8} \text{m}^2\text{s}^{-1}\text{Pa}^{-1} \). Because what is desired is a filtrate velocity (or volume flux) averaged over an entire structural unit, and because the slits only occupy a fraction \( e_s \) of the surface area, the epithelial permeability is \( k_{ep} = e_s k_s \). With the use of the representative dimensions for the rat given above, \( e_s = 0.11 \) and \( k_{ep} = 8.6 \times 10^{-9} \text{m}^2\text{s}^{-1}\text{Pa}^{-1} \). It was shown that the resistances to water flow of the zipper and ladder structures are similar, provided they are assumed to have the same ratio of wetted cylinder area to cross-sectional area (30).

Finite-element solutions of Stokes’ equation were used also to characterize the hydraulic resistance of a water-filled fenestra (31). By using the dimensions given in Lea et al. (62), the permeability of a single fenestra was estimated as \( k_f = 1.0 \times 10^{-6} \text{m}^2\text{s}^{-1}\text{Pa}^{-1} \). With the fenestrae occupying 20% of the filtering surface \( (e_f = 0.20) \), it was found that \( k_{en} = e_f k_f = 2.0 \times 10^{-7} \text{m}^2\text{s}^{-1}\text{Pa}^{-1} \). Comparing this with the epithelial result, it is found that \( k_{en}/k_{ep} \approx 20 \). This suggests that the dominant cellular contribution to \( k \) is that of the slit diaphragms and that the water flow resistance of the fenestrae is negligible. This assumes, however, that the flow resistance of the glycocalyx is unimportant (see below).

Water flow through the GBM was described by Drummond and Deen (31) using Darcy’s law

\[
\mathbf{v} = -\frac{\kappa}{\mu} \nabla P
\]

(2)

where \( \mathbf{v} \) is the local fluid velocity vector, \( \kappa \) is the Darcy permeability, \( \mu \) is the fluid viscosity, and \( P \) is the local pressure gradient. This relation is commonly used to model flow through porous or fibrous materials in situations where the pore spacings or interfiber spacings are much smaller than the dimensions of the sample. Microstructural details such as fiber concentration and fiber size are ignored, except as they influence the value of \( \kappa \) (units of \( \text{m}^2 \)). This approach is suitable when the underlying structure is complex, but pressure-flow data are available from which \( \kappa \) can be evaluated. Such data are provided by studies of filters made by consolidating isolated GBM, an approach used by Robinson and co-workers (86, 106) and by Daniels and her associates (9, 27, 34). Typical results are \( \kappa = 1–3 \text{nm}^2 \).

Equation 2 was combined with that which describes local conservation of mass (\( \nabla \cdot \mathbf{v} = 0 \)) and solved for the idealized GBM geometry shown in Fig. 1 (31). Although the actual fenestral openings are circular, a comparison of three-dimensional finite-element solutions for circular openings with two-dimensional analytical solutions for slitlike openings showed that equivalent results were obtained if the value of \( e_f \) was the same. Moreover, for the relative dimensions typical of the GBM, it was found that the infinite-series expression obtained from the analytical solution was well approximated by

\[
k_{bm} = 1 + \frac{W}{\pi L} \left( \frac{1}{n_f} \frac{3}{2} - \ln(2\pi e_f) \right) + \frac{3}{2} - \ln(2\pi e_s) \]

(3)

where \( n_f \) is the number of fenestral “slits” per structural unit. The left-hand side is the hydraulic resistance of the GBM (1/k\(_{bm}\)) relative to the resistance it would exhibit if its surface were not partially blocked by cells. The resistance of “bare” GBM is \( \mu L/\kappa \), as obtained by applying Eq. 2 to one-dimensional flow across a simple barrier of thickness \( L \). All of the terms following the “1” on the right-hand side of Eq. 3 describe the increased GBM resistance due to the fact that only parts of its upstream and downstream surfaces are accessible to filtrate. That is, the channeling of fluid flow caused by the cellular coverage has the effect of increasing the flow resistance in the GBM. In this sense, the percentages of the overall flow resistance ascribed to the cells and to the GBM are somewhat arbitrary. Although we favor simply comparing the three terms in Eq. 1, one could argue that doing so understates the cellular contribution, because both cell layers reduce \( k_{bm} \).

The main trends predicted by Eq. 3 are illustrated in Fig. 3, which shows the relative GBM resistance to water flow for various combinations of \( e_s, e_f, \) and \( n_f \). The parameter values used are those for the normal rat (Table 1). The GBM resistance in vivo is predicted to be 2.3 times that of bare GBM. Decreases in \( e_s, e_f, \) and \( n_f \) all exaggerate the channeling phenomenon, thereby increasing the water flow resistance.

Setting \( \kappa = 2.7 \text{nm}^2 \) and using the dimensions for the rat, found that \( k_{bm} = 8.3 \times 10^{-9} \text{m}^2\text{s}^{-1}\text{Pa}^{-1} \). Drummond and Deen (31). Because \( k_{bm} \approx k_{ep} \ll k_{en} \), it was
concluded that the GBM and epithelial resistances to water filtration in the normal rat are about equal and that the resistance of the endothelium is negligible. From Eq. 1, the overall hydraulic permeability was predicted to be $k = 4.1 \times 10^{-9}$ m$^2$ s$^{-1}$ Pa$^{-1}$. This is well within the range of values estimated from micropuncture measurements, which is roughly from $3 \times 10^{-9}$ to $5 \times 10^{-9}$ m$^2$ s$^{-1}$ Pa$^{-1}$ (31).

The hydraulic resistance of the GBM is proportional to $1/\kappa$ (Eq. 3), and the $\kappa$ value used above is larger than most recent estimates, including $\kappa = 1.5 \text{ nm}^2$ (34) and $\kappa = 1.2 \text{ nm}^2$ (9). Thus the GBM may actually account for somewhat more of the overall resistance than indicated. If one uses $\kappa = 1.2 \text{ nm}^2$ instead of $\kappa = 2.7 \text{ nm}^2$, the contribution of the GBM increases from 50 to 69% of the total resistance. Although the overall hydraulic permeability is then reduced by 38% to $k = 2.5 \times 10^{-9}$ m$^2$ s$^{-1}$ Pa$^{-1}$, the predicted value is still in reasonable agreement with the experimental range.

There are uncertainties also in the cellular contributions to the hydraulic permeability. The value of $k_{en}$ quoted above was computed by assuming that a fenestra is a short, water-filled channel of varying radius. An alternative model is that it is a gel-filled channel, due to the endothelial glycocalyx. When that possibility was explored by solving Brinkman’s equation (related to Darcy’s law) in a fenestra, with $\kappa = 2.7 \text{ nm}^2$ as for the GBM, $k_{en}$ was decreased to $1.3 \times 10^{-8}$ m$^2$ s$^{-1}$ Pa$^{-1}$ (31). That change alone decreases the overall hydraulic permeability from $4.1 \times 10^{-9}$ to $3.2 \times 10^{-9}$ m$^2$ s$^{-1}$ Pa$^{-1}$, with the endothelium now accounting for 24% (instead of just 2%) of the total resistance. The main obstacle to refining the estimate of $k_{en}$ is the unknown $\kappa$ of the glycocalyx.

Whereas the hydraulic resistance of the endothelium may have been underestimated, depending on the actual properties of the glycocalyx, that of the epithelium may have been overestimated. As already mentioned, the zipper structure is far too “tight” a barrier to be consistent with the relatively large test macromolecules that appear in normal glomerular filtrate. Larger openings in the slit diaphragm would also tend to increase the value of $k_{ep}$. To refine models either for water flow or for macromolecule movement through the filtration slits, an improved representation of the slit diaphragm geometry is needed.

Uncertainties in the individual contributions notwithstanding, the success of the water flow model in predicting the overall hydraulic permeability suggests that the overall balance between the GBM and cellular resistances is approximately correct. Indeed, the tendency to underestimate the endothelial contribution may well have canceled a tendency to overestimate the epithelial contribution. In most of the applications to pathophysiological situations described below, the fenestral and slit diaphragm permeabilities are each assumed to be constant, and the main factor considered is the calculated change in $k_{bm}$. Under those conditions, precisely apportioning the cellular resistance between the two layers is much less important than describing the effects of the cells on $k_{bm}$.

Applications of Water Flow Model to Glomerular Disease

The first pathophysiological application of the water flow model was to adriamycin nephrosis in the rat (31). The morphometric and micropuncture results used were those of Miller et al. (69), who studied the effects of adriamycin administration in three groups of animals: group 1, no further treatment; group 2, four-fifths renal ablation; and group 3, low-protein diet. Relative to the values quoted above for normal rats, $W$ was increased by factors of 5–7 (reflecting decreases in measured filtration slit frequency), and $L$ was increased by factors of 1.5–2.5 (reflecting measured values of basement membrane volume divided by peripheral capillary surface area). Another prominent finding was the detachment of foot processes from as much as 4% of the capillary wall. That was modeled by considering two parallel pathways for water filtration, one with all structures present and the other with $\xi_s = 1$ and $k_{ep} = \infty$. To examine the possible consequences of slit diaphragm disruption in areas with intact foot processes, calculations were performed with the “normal” value of $k_{en}$ given above or with $k_{en} = \infty$. The normal value of $k_{en}$ was used in each case, and the hydraulic permeabilities of the two pathways were weighted according to their respective area fractions. For groups 1 and 2, the value of $k$ determined by micropuncture was in the middle of the range of the predicted values. For group 3, the experimental value closely matched the prediction using the normal $k_{en}$, suggesting that the reduction in glomerular volume associated with the low-protein diet may have attenuated the rupture of slit diaphragms.

The model has been applied also to human glomerular disease. In each of the four diseases studied to
date, impairment of $k$ appears to be the predominant cause of glomerular filtration rate (GFR) depression early in the course of the disorder. The conditions examined include minimal change, membranous, and diabetic nephropathies, and preeclamptic toxemia (33, 58, 79, 97). In each instance depression of GFR by 30–50% was associated with alterations in glomerular hemodynamics that should not have reduced the net ultrafiltration pressure and hence the GFR. By exclusion, we infer that GFR depression must have been due to a decline in the ultrafiltration coefficient ($K_f$). $K_f$ is the product of glomerular hydraulic permeability and filtration surface area ($K_f = kS$), expressed either on a single-nephron or whole-kidney basis; single-nephron values are employed here.

In the human studies to be discussed, glomeruli obtained by biopsy were subjected to morphometric analysis to determine $L$, $F$S (allowing calculation of $W$), filtration surface area per glomerulus ($S$), and certain other quantities. The value of $S$ was computed from the product of filtration surface density and glomerular volume (33, 97). Except where indicated, the values of parameters employed in the water flow model ($k_{en}$, $e$, $n_f$, $k$, $s$, $w$) other than $L$ and $W$ were assumed to be the same as the original set used for normal rats (31), as given above. Control values of $L$ and $W$ were provided by groups of subjects with normal glomeruli (living kidney transplant donors). In the controls and in three forms of glomerular injury (diabetic, minimal change, and membranous nephropathy), transmission electron micrographs showed large and numerous endothelial fenestrae, and the endothelial resistance to water flow was neglected. In membranous (33, 97), minimal change (33), and diabetic nephropathy (79), the main contribution to the reduction in $k$ was found to be the increase in $W$. In preeclamptic toxemia, an observed reduction in the size and number of fenestrae made the calculated endothelial contribution important (58).

An example is provided by findings in a group of 15 patients with membranous nephropathy. Each had a severe glomerular injury characterized by persistent nephrosis and a progressive decline in GFR over a 2- to 5-yr period of observation (97). Glomerular structure and ultrafiltration capacity were examined on two occasions, at the time of presentation and diagnostic biopsy (baseline) and again after 2–5 yr. At baseline, glomerular volume was larger than control, and it was estimated that $S$ increased by some 40%. Membranous nephropathy at this time was accompanied by an approximate doubling of $L$ and a roughly fourfold increase in $W$, reflecting a marked widening of both the GBM and the epithelial foot processes. Using Eqs. 1 and 3, it was found that there was a marked depression of $k$, $0.79 \pm 0.09 \times 10^{-9} \mathrm{m} \cdot \mathrm{s}^{-1} \cdot \mathrm{Pa}^{-1}$ in membranous nephropathy vs. $2.8 \pm 0.09 \times 10^{-9} \mathrm{m} \cdot \mathrm{s}^{-1} \cdot \mathrm{Pa}^{-1}$ in controls. The corresponding values of $K_f$ predicted by the model were $3.4 \pm 0.7 \mathrm{nl} \cdot \mathrm{min}^{-1} \cdot \mathrm{mmHg}^{-1}$ in membranous nephropathy and $7.1 \pm 0.6 \mathrm{nl} \cdot \mathrm{min}^{-1} \cdot \mathrm{mmHg}^{-1}$ in controls. This estimated 52% reduction in $K_f$ was sufficient to account for the observed reduction in GFR ($56 \pm 8$ vs. $102 \pm 2 \mathrm{ml} / \mathrm{min} / \mathrm{m}^2$).

The later analysis (2–5 yr beyond baseline) revealed no further changes in $F$S (or $W$), but there were increases in $L$ to roughly four times that of control and reductions in $S$ to $\sim 30\%$ below control values. The persistent nephrosis was associated with an additional, significant decline in GFR in each individual. Because $k$ at this later time was computed to be not significantly different from that at baseline, it was concluded that the further reduction in GFR was attributable entirely to the reduced $S$. To summarize, the serial observations permit the conclusion that progressive hypofiltration in membranous nephropathy is a consequence of a biphasic loss of glomerular filtration capacity, consisting of an initial reduction in $k$ that is later exacerbated by a loss of $S$ (97).

Given that the GBM is a significant contributor to the overall water flow resistance, one might expect that the doubling of GBM width between biopsies in the membranous nephropathy patients would have lowered $k$ even further below that of controls. However, with a very low FSF, it was assumed in this group that despite approximately twofold larger values of $L$ in membranous nephropathy. Because the measured values of $S$ and of the hemodynamic determinants of GFR did not differ greatly, this explained the similar values of GFR in the two groups.

A group of glomerular diseases that fit loosely into the category of "thrombotic microangiopathy" or "hemolytic uremic syndrome" can lower GFR while having no discernable effect on the GBM or epithelial foot processes. Rather, this group of glomerulopathies is associated with substantial injury to glomerular endothelial cells. In subjects with preeclamptic toxemia, which is an example of a thrombotic microangiopathy, GFR was found to be depressed by 39% relative to healthy gravid controls (58). Reductions in filtration surface density due to mesangial interposition were partially offset by glomerular hypertrophy, resulting in values of $S$ that tended to be slightly lower than in controls. Neither GBM thickness nor FSF was altered, but there were extensive, dense, subendothelial deposits of fibrinoid material that substantially lengthened the filtration pathway (from fenestral interface to slit diaphragm). The circumferential rim of endothelial cytoplasm was characterized by swollen segments that were devoid of fenestrae. A morphometric analysis of "en face" sections of endothelium by scanning electron microscopy revealed that $e_f$ was drastically reduced, from 0.16 in controls to 0.014–0.087 in the subjects with preeclamptic toxemia. The fenestrae were also
smaller, as evidenced by a reduction in their area-to-perimeter ratio to one-half that of controls. From this structural information, it was estimated that \( k \) was reduced by \( \sim 30\% \) in preeclamptic toxemia. Taken together with the trend toward lower \( S \), it was calculated that \( K_f \) was likely to have been depressed by \( \sim 40\% \) in preeclamptic toxemia, similar to the reduction in GFR.

**GBM Nanostructure and \( \kappa \)**

The value of \( \kappa \) of a fibrous membrane or gel can be evaluated by using Eq. 2 to interpret measurements of fluid velocity as a function of applied pressure, as was done in deriving the values for rat GBM used above. The value of \( \kappa \) can also be predicted, in principle, from nanostructural information. Numerous theoretical results are available to predict \( \kappa \) for media consisting of arrays of cylindrical fibers with fluid-filled interstices; in some, the fibers are assumed to have a regular, spatially periodic arrangement, whereas in others the fiber orientation is random. The results of several approaches are reviewed in Jackson and James (46). More recent results for random arrays of fibers include those of Clague and Phillips (17) and Clague et al. (16).

A model developed specifically for the GBM is that of Palassini and Remuzzi (80), who adopted a tetrahedral fiber arrangement, based on the structure of collagen IV. The application of several theories to GBM is discussed in Bolton and Deen (8). For fibers of uniform radius \( (r_f) \), the results for regular or random arrays are typically of the form

\[
\frac{k}{r_f^1} = f(\phi)
\]

where \( \phi \) is the volume fraction of fibers, and the theory provides the specific function \( f(\phi) \), which always decreases as \( \phi \) increases. This implies that, for a fibrous material with a specified solids content, \( \kappa \propto r_f^2 \). In other words, the predicted value of \( \kappa \) is extremely sensitive to the value chosen for the fiber radius.

With \( \phi \approx 0.1 \), as has been reported for GBM (21, 85), realistic values for \( \kappa \) (in the range of 1–2 nm\(^2\)) are obtained from any of the theoretical expressions if the fiber radius is assumed to be \( \sim 1 \) nm (8). However, if \( r_f = 3–4 \) nm is employed, corresponding to the radii of fibers visible in electron microscopic images, the predicted value of \( \kappa \) is an order of magnitude too large. This led to the suggestion that GBM be modeled as a mixture of coarse and fine fibers, the former corresponding roughly to collagen IV fibrils and the latter to glycosaminoglycan chains (8, 34). Underlying this suggestion is the presumption that the fine fibers would not have been resolved in the electron micrographs. With coarse and fine fiber radii of 3.5 and 0.5 nm, respectively, and roughly a 1:1 mixture (by volume) of the two fiber types, it was possible to reconcile the measured values of \( \kappa \) and \( \phi \) with the electron microscopic appearance of GBM. Parameter values for this two-fiber model of the GBM, which should be viewed as quite tentative, are summarized in Table 2.

| Radius of coarse fibers, \( r_1 \), nm | 3.5 |
| Radius of fine fibers, \( r_2 \), nm | 0.5 |
| Volume fraction of coarse fibers, \( \phi_1 \) | 0.046 |
| Volume fraction of fine fibers, \( \phi_2 \) | 0.054 |
| Total volume fraction of solids (fibers), \( \phi \) | 0.10 |

Additional quantitative information on the composition and the spatial arrangement of proteins and proteoglycans would be invaluable in efforts to reach more definite conclusions about the nanostructural basis for \( \kappa \) in the GBM. Analogous information is needed to estimate \( \kappa \) in the endothelial glycocalyx and thereby better define the endothelial resistance to water flow.

**Filtration of Macromolecules**

**General Relationships**

This section begins with a discussion of physical phenomena that underlie efforts to relate macromolecule permeability to the structure of the glomerular capillary wall. Several key quantities are defined. In keeping with the microscopic viewpoint adopted for water filtration, this discussion focuses on the local sieving coefficient, which is the filtrate-to-plasma concentration ratio at a particular point along a capillary. This must be distinguished from the sieving coefficient for a whole kidney (or representative capillary), which is the average concentration in Bowman’s space divided by that in afferent plasma. It is the average sieving coefficient that is accessible experimentally (e.g., from the fractional clearances of exogenous tracers). Even if the structure of the capillary wall is uniform along its length, the local sieving coefficient will vary with position, mainly because of the progressive increase in plasma protein concentration from the afferent to the efferent end. It has long been recognized that the resulting increase in oncotic pressure along a capillary will tend to slow filtration, which in turn will affect local sieving. Proteins may also have other effects on barrier performance, as will be discussed. The calculation of the average (measurable) sieving coefficient from local solute and volume fluxes (generally not measurable) has been described (e.g., Ref. 66). Although the local and average sieving coefficients are not identical, factors that affect the former will have a qualitatively similar influence on the latter.

The relationship between the overall sieving coefficient at any position along a capillary (\( \Theta \)) and those of the individual layers can be approximated as

\[
\Theta \approx \Theta_{en} \Theta_{bm} \Theta_{ep}
\]

For example, \( \Theta_{bm} \) is the concentration at the downstream edge of the GBM divided by that at the upstream edge, with both concentrations evaluated just inside the GBM. To the extent that \( \Theta_i \rightarrow 1 \) for layer \( i \), that layer will not contribute to the observed selectivity of the barrier. It is important to note, though, that the product in Eq. 5 implies that a 10% change in any
individual $\Theta_i$ will affect the overall $\Theta$ by the same 10%, whether layer $i$ is highly selective (e.g., $\Theta_i = 0.001$) or not (e.g., $\Theta_i = 0.9$). This contrasts with the situation for water flow, where the additive series-resistance relationship (Eq. 1) implies that if layer $i$ contributes a negligible fraction of the overall resistance (i.e., if $1/k_i \ll 1/k$), then a 10% change in $k_i$ will have no noticeable effect on $k$. Thus the layers combine to influence macromolecule selectivity in a fundamentally different way than they combine to influence water filtration. To obtain a more precise relationship between the overall $\Theta$ and those of the individual layers, additional factors must be included in Eq. 5 to account for the effects of soluble proteins (e.g., albumin) on the equilibrium partitioning of macromolecules (61).

Another important distinction between water filtration and macromolecule sieving is that the individual $\Theta_i$ values affect one another, whereas the individual $k_i$ values could be computed independently. Moreover, the $\Theta_i$ values depend in general on the filtrate velocity, whereas the $k_i$ values could be approximated as constants. (Constancy of $k$ assumes, of course, that the applied pressures are not so large as to alter the structure of the capillary wall). The interdependence of the layer sieving coefficients and the effects of filtrate velocity are illustrated next by a somewhat simplified model for transport in the GBM. As discussed later, an extension of that approach is a central feature of a model for transport in the GBM. As discussed later, an extension of that approach is a central feature of a structure-based model that has been proposed to describe glomerular size selectivity.

As in the application of Darcy’s law (Eq. 2), the GBM will be regarded as an isotropic medium, such as an array of randomly oriented fibers. In such a material the local flux ($N$) of an uncharged macromolecule may be expressed as

$$N = -K_d D_v C + K_v C$$  (6)

where $D_v$ is the solute diffusivity in free solution, $v$ is the local fluid velocity vector, $C$ is the solute concentration, and $K_d$ and $K_v$ are hindrance factors for diffusion and convection, respectively. The local solute concentration is based here on total volume (water plus solids), as is usually done in describing equilibrium partitioning or transport in gels. Just as Eq. 2 relates the local fluid velocity to the pressure gradient, Eq. 6 relates the local solute flux to the concentration gradient and the fluid velocity.

The diffusivity and hindrance factors in Eq. 6 all depend on molecular size. The standard measure of molecular size is the Stokes-Einstein radius ($r_e$), because knowing it is equivalent to knowing $D_v$. For a spherical molecule of radius $r_e$ in water at 37°C, the relationship is $D_v = (3.28 \times 10^{-5} \text{ cm}^2/\text{s})/r_e$ (where $r_e$ is in Å). In general, steric and hydrodynamic interactions between a macromolecular solute and the fixed polymeric fibers of a membrane or gel will cause $K_d$ and $K_v$ to be less than unity, with both decreasing as $r_e$ increases. The experimental estimation of these hindrance factors in GBM is discussed later. Another property of a fibrous membrane or gel that influences transport and depends on $r_e$ is the equilibrium partition coefficient ($\Phi$). The partition coefficient is a thermodynamic quantity that describes the tendency of steric and/or electrostatic interactions to exclude macromolecules from the material. As with the hindrance factors, it is typically less than unity and decreases with increasing $r_e$. As defined here, if the GBM were in equilibrium with plasma, then $C = \Phi C_p$, where $C_p$ is the plasma concentration. Steric exclusion from the GBM is important, but it appears that electrostatic interactions are not (9). Although the partition coefficient does not appear in Eq. 6, it enters the analysis when concentrations within the GBM are related to those in plasma or the other structures.

Assume for the moment that the GBM extends from $z = 0$ to $z = L$, that the solute concentration depends only on $z$, and that the solute flux and fluid velocity (magnitudes $N$ and $v$, respectively) are each constant. This “one-dimensional” model, involving just $z$, corresponds to a hypothetical GBM with fully accessible surfaces (i.e., $\epsilon_s = \epsilon_e = 1$). As will be seen later, only a slight modification of the results is needed to describe the more realistic situation where the surfaces are largely blocked by cells. In the one-dimensional model, the solute concentration profile in the GBM can be derived analytically for any specified values of $\Theta_{en}$ and $\Theta_{ep}$. This allows the sieving coefficient in the GBM to be evaluated. The result is

$$\Theta_{bm} = \frac{\Phi K_e}{\Theta_{ep}(1 - e^{-Pe}) + \Phi K_e e^{-Pe}}$$  (7)

where $Pe$ is the Péclet number

$$Pe = \frac{(\Phi K_v) v L}{(\Phi K_d) D_v}$$  (8)

Notice in Eq. 7 that the sieving coefficient in the GBM depends on that for the epithelium (filtration slits). Notice also the effect of $v$, which is in the numerator of $Pe$ (Eq. 8). The physical significance of the Péclet number is that it measures the importance of convection relative to diffusion; convection tends to dominate for large $Pe$ and diffusion for small $Pe$. Equation 8 has been written with the common factor $\Phi$ in the numerator and denominator to emphasize that, because only the products $\Phi K_e$ and $\Phi K_d$ appear there and in Eq. 7, those two lumped quantities are sufficient to describe the intrinsic size selectivity of a membrane such as the GBM. That is, $\Phi$, $K_e$, and $K_d$ need not be known separately. Although the simplified model employed here assumes that $\Phi$ for a tracer (e.g., Ficoll) has the same value at both sides of the GBM, a more detailed theory indicates that it depends on the local concentration of albumin and other abundant proteins (61). Accordingly, it is expected to differ at the two sides of the GBM, as discussed later.

The dependence of $\Theta_{bm}$ on $\Theta_{ep}$ predicted by Eq. 7 is illustrated by the curve labeled “1-D model” in Fig. 4. In these calculations $Pe$ and $\Phi K_e$ were held constant at values representative of a macromolecule with $r_e = 35$ Å in rat GBM. It is seen that $\Theta_{bm}$ is predicted to range from values above unity for a highly selective filtration
slit ($\Theta_{ep} \to 0$) to values below unity for a nonselective one ($\Theta_{ep} = 1$). The behavior for highly selective slits reflects concentration polarization within the GBM, as noted in Edwards et al. (35). That is, a concentration increase in the direction of flow arises to provide a diffusional driving force in the other direction. The opposing contributions of diffusion and convection in the GBM reduce $N$ to what can be accommodated by the slit, thereby maintaining the steady state. Inspection of Eq. 7 reveals that the upper limit of the polarization effect in the GBM is $\Theta_{bm} \to \exp(Pe)$ for $\Theta_{ep} \to 0$. It is also seen that GBM polarization disappears exactly (i.e., $\Theta_{bm} = 1$) if $\Theta_{ep} = \Phi K_c$, for any $Pe$. Only for $\Theta_{ep} > \Phi K_c$ is the slit permeable enough to allow the basement membrane to enhance the overall selectivity (i.e., $\Theta_{bm} < 1$), rather than degrade it. A final noteworthy aspect of Eq. 7 is that it shows that $\Theta_{bm} \to 1$ as $Pe \to 0$, for any positive values of $\Theta_{ep}$ and $\Phi K_c$. This is an example of a well-known phenomenon in ultrafiltration, which is the tendency for filtrate and retentate concentrations to equilibrate as diffusion becomes more important. In this instance, the equilibration is just across the GBM.

The simplified, one-dimensional analysis just discussed illustrates an important, general point, which is that the individual sieving coefficients depend on one another and on the relevant Péclet number(s). Although the Péclet number discussed was that for the GBM, analogous Péclet numbers for the fenestrae and filtration slits can be expected to influence $\Theta_{en}$ and $\Theta_{ep}$, respectively. Such effects have been discussed in models of the slit diaphragm (32, 35). A consequence of the dependence of the sieving coefficient on the Péclet numbers is that great care must be taken in extrapolating results from one experimental situation to another. For example, one cannot expect a sieving coefficient measured for GBM in vitro to equal that in vivo, even if the isolated GBM preparation is perfect. The thickness of a filter made by consolidating GBM fragments will greatly exceed that of a single layer of GBM and the filtrate velocity is unlikely to equal that in vivo; both of these differences will affect $Pe$ (Eq. 8). Moreover, the modifying effect of the epithelial sieving coefficient will be absent.

**Experimental Assessment of GBM and Cellular Contributions**

As mentioned earlier, measurements of water filtration rates across filters prepared from isolated GBM have permitted the evaluation of its $\kappa$. Sieving experiments using similar isolated GBM preparations have been valuable in assessing its selectivity to macromolecules, including proteins, neutral and charged derivatives of dextrans, and neutral and charged derivatives of Ficoll (9, 18, 19, 25, 27, 34, 106). Ficoll has been preferred in the more recent studies, because it diffuses as an ideal, neutral sphere (7, 28) and because it can be used also in fractional clearance studies in vivo (e.g., Refs. 6, 75, and 83). An example of sieving data obtained in isolated rat GBM with uncharged Ficoll is shown in Fig. 5. The data are those of Bolton et al. (9), as replotted in slightly modified form by Lazzara and Deen (61). As shown by the lower set of symbols, which are results for protein-free solutions, there was a gradual decline in sieving coefficient with increasing molecular size, from $-0.6$ at $r_s = 20$ Å to $-0.03$ at $r_s = 50$ Å. Not shown in Fig. 5 are results obtained for Ficoll sulfate, which were indistinguishable from those for Ficoll (9).

The sieving results for Ficoll and Ficoll sulfate in protein-free solutions were analyzed by Lazzara and Deen (61) to estimate values of $\Phi K_d$ and $\Phi K_c$ for GBM. The data were fitted using a sieving relationship sim-
similar to Eq. 7 (but with $\Theta_{ep} = 1$) and assumed expressions of the form

$$\Phi K_d = \exp(-Ar_s)$$

(9)

$$\Phi K_c = \exp(-Br_s)$$

(10)

The values of the empirical constants $A$ and $B$ were very similar for Ficoll and Ficoll sulfate, with averages of $A = 0.130 \text{ Å}^{-1}$ and $B = 0.072 \text{ Å}^{-1}$ for the two sets of data. Equations 9 and 10 have no theoretical basis, except for the expectation that both quantities should be near unity for small $r_s$ and should decline to zero for very large molecules. Nonetheless, as shown by the lower curve in Fig. 5, excellent fits to the data for $20 \leq r_s \leq 50 \text{ Å}$ were obtained with just the two adjustable parameters. Empirical expressions similar to Eqs. 9 and 10 were also employed previously (9, 35).

The use of Eqs. 9 and 10 to make inferences about the glomerular capillary wall assumes, of course, that the isolated GBM was not functionally different from that in vivo. The possibility that GBM is altered during the isolation process has been examined using a variety of methods. Immunofluorescent microscopy of consolidated GBM filters demonstrated the presence of type IV collagen, laminin, and the core protein of heparan sulfate proteoglycan (27), the main components of GBM. The sulfated side chains of GBM proteoglycans are also present in GBM isolated using $N$-lauryl sarcosine to lyse cells (25), the procedure employed to obtain the data from which Eqs. 9 and 10 were derived (9). The permeability of GBM filters was not changed when a milder detergent, Triton X-100, which has been shown to preserve heparan sulfate proteoglycan, was used to lyse glomerular cells (25). That isolated GBM is relatively intact is suggested also by electron microscopy studies: the spatial distribution of cationic ferritin has been found to be similar to that in vivo (55).

A technical advance due to Daniels and co-workers (26, 36) that has permitted the measurement of diffusional permeabilities for macromolecules is the use of confocal microscopy to monitor the movement of fluorescent tracers across segments of isolated glomerular capillaries. Experiments have been performed with intact glomeruli, freshly isolated from rats, and with glomeruli in which the cells have been removed by detergent lysis, leaving only GBM. Thus it has been possible to compare the diffusional permeability of intact capillary walls ($p$) with that of bare GBM ($p_{bm}$). Diffusional permeabilities of series barriers obey a resistance formula like Eq. 1, so that

$$\frac{1}{p} = \frac{1}{P_{en}} + \frac{1}{P_{bm}} + \frac{1}{P_{ep}} = \frac{1}{P_{bm}} + \frac{1}{P_{cell}}$$

(11)

The two cellular contributions, which cannot be distinguished using this approach, have been lumped together in the second equality as $P_{cell}$. Edwards et al. (36) measured $p$ and $p_{bm}$ for four narrow fractions of Ficoll ($r_s = 30–62 \text{ Å}$) and found that $p_{bm}$ for each molecular size was an order of magnitude larger than $p$. It was calculated that the GBM contributes only 13–26% of the diffusional resistance of the intact capillary wall (depending on $r_s$). The finding that $P_{cell} \ll P_{bm}$ for Ficoll is qualitatively similar to earlier results for dextran (26).

The experimental estimates of the GBM hindrance factors for Ficoll are plotted in Fig. 6. The results for $\Phi K_d$ and $\Phi K_c$ derived from sieving data (Eqs. 9 and 10) are compared with values of $\Phi K_d$ calculated from $P_{bm}$. The relationship between the diffusional permeability and diffusional hindrance factor is $p_{bm} = \Phi K_dD_m/L$, where $L$ (the GBM thickness) was taken to be 200 nm. The agreement between the two independent estimates of $\Phi K_d$ is remarkably good, given the different experimental preparations and the several assumptions required in making this comparison. The finding that $\Phi K_c \gg \Phi K_d$ for Ficoll is qualitatively consistent with data for globular proteins and Ficoll in agarose gels (49, 52, 53).

Using $v = 4 \mu\text{m/s}$ as a typical average filtrate velocity for the rat (corresponding roughly to single-nephron GFR = 40 nl/min), $P_e$ calculated from Eqs. 8–10 ranges from 0.016 at $r_s = 20 \text{ Å}$ to 0.22 at $r_s = 50 \text{ Å}$. These small values of $P_e$ indicate that diffusion within the GBM is relatively rapid in vivo (compared with convection), even for relatively large molecules. A consequence of this is that concentration polarization within the GBM will tend to be minimal, even if the filtration slits are highly selective barriers. This tends to mitigate objections that are sometimes made to a glomerular capillary “design” in which the limiting barrier is the one farthest downstream. Although diffusion in the GBM is rapid relative to convection, it is still much slower than diffusion in water. This is indicated by the small values of $\Phi K_d$ in Fig. 6. For example, $\Phi K_d = 0.01$ (the value for $r_s = 35 \text{ Å}$) means that the diffusional permeability of

![Fig. 6. Diffusive ($\Phi K_d$) and convective ($\Phi K_c$) hindrance factors for Ficoll in GBM as a function of $r_s$. Values of $\Phi K_d$ calculated from the confocal microscopy data of Edwards et al. (36); solid lines, the estimates from sieving data in isolated GBM without BSA (Eqs. 9 and 10); dashed curves include the predicted effect of BSA on $\Phi$; increases in $\Phi$ due to a BSA concentration of 6.2 g/dl at the upstream side of the membrane were computed as in Ref. 61 and Eqs. 9 and 10 modified accordingly.](http://ajprenal.physiology.org/)
the GBM is only 1% of that of a film of water of equivalent thickness.

Not considered in Fig. 6 are the possible effects of GBM compressibility on macromolecule partition coefficients and diffusive or convective hindrance factors. In particular, the sieving data used were obtained at an applied pressure of ΔP = 60 mmHg (9), whereas the diffusion experiments (36) corresponded to ΔP = 0. The hydraulic permeabilities and/or κ values of filters made from isolated GBM have been found to decrease with increases in applied pressure (ΔP) (27, 34, 86, 106). Because f(ϕ) in Eq. 4 decreases with increasing ϕ, one would expect κ to decrease if compression of the GBM forces water out and thereby increases the volume fraction of solids. On the basis of theories for fiber matrices, increases in f are expected to also result in decreases in Φ (60, 71) and Kf (49, 81). Experimental results for proteins and Ficoll in agarose suggest that Kf would decrease as well (52, 53). Attempts have been made to model the effects of pressure on ΦKd and ΦKc (34, 35), but these efforts are confounded by the lack of an adequate theory for Kc in fibrous materials and by the probable effects of BSA on the values of Φ for Ficoll (61). The effects of BSA are an issue because BSA has been present in some sieving experiments with isolated GBM, but not others.

The interpretation of p_{cell} depends, of course, on the relative contributions of the endothelium and epithelium to the diffusional resistance of the intact capillary wall. Assuming that the cellular resistance resides in the slit diaphragm, and modeling that structure as a row of parallel cylinders (as in the “ladder” of Fig. 2), Edwards et al (36) found that the diffusion results could be explained by a cylinder spacing that followed a lognormal distribution, with small areas (~0.2%) devoid of cylinders. That representation of the cellular barrier was incorporated into later simulations of macromolecule filtration in vivo (35). The one significant difference was that in healthy subjects, at least, there was no evidence for “shunts” created by small areas of the slit diaphragm devoid of cylinders.

As already stated, it was found that sieving curves measured in isolated GBM for Ficoll and its anionic derivative, Ficoll sulfate, were indistinguishable. Only when the ionic strength of the solutions was reduced below physiological levels, thereby amplifying the effects of electrostatic interactions, was Θ_{bm} for Ficoll sulfate less than that of neutral Ficoll (9). This finding of little or no charge selectivity is generally consistent with other studies of isolated GBM. That is, Bray and Robinson (11) found only small differences in sieving curves for dextran and dextran sulfate (DS), and Bertolatus and Klinzman (5) noted only small differences in the filtration rates of native (anionic) and cationized BSA. Procedures used in those laboratories to neutralize GBM charge, including methylation of carboxyl groups (5) and reductions in pH from 7.4 to 5.7 (the isoelectric point of GBM) (85) had little effect on the sieving of BSA. Similarly, Daniels (25) found that treating the GBM with heparitinase to remove heparan sulfate proteoglycan, adding protamine to neutralize GBM polyanions, or reducing the experimental pH to the isoelectric point of the GBM or BSA had little or no effect on the sieving coefficient of BSA. Thus to the extent that the glomerular barrier is charge selective, it is the cellular layers, and not the GBM, which appear to be responsible. The charge selectivity of the intact glomerular capillary wall is discussed below.

Structure-Based Model for Size Selectivity

With the structural unit depicted in Fig. 1 serving as a framework, and using experimental information on the transport of Ficoll in isolated GBM and intact capillaries (9, 34, 36), Edwards et al. (35) constructed a model for the filtration of uncharged macromolecules in vivo. The concentration field within the GBM was computed by combining Eq. 6 with the steady-state form of the solute conservation equation (V·N = 0) and appropriate boundary conditions and solving for C(x,z) using a finite-element method. The results of solutions computed with many different combinations of the geometric parameters were found to be correlated accurately by replacing Pe in Eq. 7 by Pe*, where

\[
\frac{Pe^*}{Pe} = 1 + a(1 - \epsilon_r \epsilon_b)\left(\frac{L}{W}\right)^{-c}
\]

and a = 0.7366, b = 11.9864, and c = 1.2697. For the baseline parameters in Table 1, Pe* = 2.2 Pe. Thus the partial blockage of its surfaces by endothelial cells and foot processes is predicted to approximately double the apparent value of the Pecllet number in the GBM. The values of Pe* remain relatively small, however, and the predicted dependence of Θ_{bm} on Θ_{ep} remains much the same as that for the one-dimensional model. This is shown by the curve labeled “2-D model” in Fig. 4. The factor by which the apparent Péclet number is increased relative to “bare” GBM is almost identical to the factor by which Θ_{bm} is decreased (2.2 vs. 2.3). Both effects may be interpreted as the result of an increase in the effective path length for the filtrate, caused by the diverging-converging flow pattern.

The hypothesis explored by Edwards et al. (35) is that the structure responsible for the cellular barrier to uncharged macromolecules is the slit diaphragm. As already mentioned, the slit diaphragm was modeled as a single row of cylindrical fibers of equal radius but nonuniform spacing. The advantages of postulating a nonuniform spacing were pointed out by Drummond and Deen (32), who developed a hydrodynamic model for the hindered transport of macromolecules through a single row of cylindrical barriers. If a lognormal distribution of spacings is adopted for the “ladder rungs” in Fig. 2, then many of the fiber spacings would be close to the dimensions reported in Rodewald and Karnovsky (87). Consequently, the hydraulic permeability can be made to match that of the zipper structure. Although infrequent, the larger spacings allow sufficient passage of large molecules to be consistent with diffusional results for Ficoll in isolated glomeruli (36) and fractional clearances of Ficoll measured in vivo (6, 75, 83). If the 40 A wide openings of Rodewald

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and Karnovsky (87) applied throughout the slit diaphragm, then no molecule with $r_s > 20$ Å would enter the filtrate, whereas Ficolls with $r_s > 60$ Å are readily detectable in normal urine (6, 75, 83). Thus the lognormal distribution of spacings was an attempt to reconcile the electron microscopic appearance of the slit diaphragm with fractional clearance data in vivo.

In that the most restrictive part of the barrier was assumed to be the slit diaphragm, it is not surprising that the sieving coefficients predicted by the model of Edwards et al. (35) were very sensitive to the values chosen for the fiber radius and the fiber spacing parameters. Parameter values were found that provided very good fits to Ficoll data in normal rats (75, 83) and healthy humans (6), although the discrepancies in the fractional clearances themselves precluded identification of a single set that would closely match the results of any two studies. As already mentioned, it was unnecessary to postulate a shunt in the slit diaphragm in vivo. The values of the GBM hindrance factors were found to be important mainly as they influenced $P_e$; that is, the ratio $K_c/K_d$ was much more influential than either $\phi K_d$ or $\phi K_s$ individually. This is a consequence of the behavior of Eq. 7 for $P_e < 1$ and $\Theta_{ep} \ll \phi K_c$, conditions that held in most of the simulations.

Simulations of the effects of hemodynamic perturbations on sieving coefficients provide a clue that the model for size selectivity may need to be modified. That is, selective perturbations in afferent plasma flow rate ($Q_\Lambda$) were found to have almost no effect on the average (observable) sieving coefficients (35). This is in contrast to predictions for a one-layer capillary wall, where the average $\Theta$ for a capillary decreases with increasing $Q_\Lambda$ as a result of both the higher single-nephron GFR and lower filtration fraction for water (66). The somewhat surprising result for the composite barrier is explained by a tendency for increased concentration polarization in the GBM to cancel the other effects (35). Thus examining the dependence of sieving curves on $Q_\Lambda$ may provide insight into the amount of concentration polarization actually present. There are no such data for Ficoll, but increasing $Q_\Lambda$ in rats by plasma volume expansion was found to decrease the fractional clearances of dextrans (15). This suggests that for dextran, at least, concentration polarization within the GBM may be absent. This raises the possibility that the glycocalyx-filled fenestrae may have a greater role in size selectivity than has been supposed, and the slit diaphragm a lesser role. Similar experiments with Ficoll, a more ideal test molecule than dextran, might clarify the situation. In addition to the tentative nature of the assumption that $\Theta_{en} \approx 1$, the model of Edwards et al. (35) does not fully incorporate recent findings concerning steric effects of plasma proteins on the partitioning and sieving of tracers (see below). Finally, that model was intended only to describe glomerular size selectivity, and not charge selectivity.

**Charge Selectivity Data**

Research in the 1970s and 1980s led to the view that the glomerular capillary wall discriminates among macromolecules on the basis of their net charge as well as their size (66). The pattern seen was that, for a given $r_s$ and molecular conformation, anionic polymers passed through the capillary wall less readily than did neutral polymers, which in turn passed less readily than cationic polymers. Differences due to molecular charge tended to be diminished in proteinuric disorders. The inference was that fixed negative charges in one or more parts of the capillary wall normally make entry into and passage through the barrier less favorable for polyanions (such as albumin) than for neutral molecules of similar size and configuration. Much of the evidence for charge selectivity was based on comparisons between the fractional clearances in rats of dextran (uncharged) and DS (anionic) (e.g., Ref. 14). Other influential studies employed native (neutral) and anionic horseradish peroxidase (nHRP and aHRP, respectively) (e.g., Ref. 84). Technical concerns have been raised in recent years concerning both sets of test molecules, motivating a reexamination of the concept of charge selectivity. Indeed, arguments against glomerular charge selectivity are the main theme of a review by Comper and Glasgow (20). What follows is a summary of certain key issues and a review of the most recent findings.

At least two factors may complicate the interpretation of fractional clearance data for DS. First, it has been shown that DS binds to plasma proteins (41, 68). This binding was studied extensively by Guasch et al. (41), using ultrafiltration and equilibrium dialysis experiments with $^3$H-DS and/or unlabeled DS added to Krebs buffer solutions or to human serum. For the relatively small sizes of $^3$H-DS examined, only some 45% of the activity in serum was not protein bound. Use of total radioactivity to determine the plasma concentration of a protein-bound tracer will tend to overestimate the concentration of free tracer and therefore underestimate its urinary clearance. Nonetheless, when corrections were made for protein binding, the fractional clearance of $^3$H-DS with $r_s = 15–18$ Å in normal rats (68) or humans (41) was still only 0.5–0.7, much smaller than that for dextran of similar size ($=1$). This charge selectivity was almost abolished in the nephrotic syndrome, the fractional clearance of $^3$H-DS increasing from 0.68 in healthy humans to 0.95 in nephrotic patients (41).

Another concern with the use of DS is cellular uptake and intracellular desulfation, as examined in a series of studies by Comper and co-workers (12, 13, 22, 100, 104, 105). When $^3$H-DS was added to isolated kidney perfusates or administered intravenously to rats, most of the tritiated polymer in urine was found to be desulfated (12, 22, 104). This occurred without a significant change in molecular size (12, 22). Evidence was found for uptake of $^3$H-DS, but not uncharged dextran, by glomerular cells (100, 105), and it was argued that the glomerulus is a primary site for de-
The criticism of the data with HRP is based on the finding that aHRP is preferentially degraded in the kidney (76). Accordingly, the use of an enzymatic assay to detect aHRP in kidney tissue and urine leads to a systematic underestimate of its sieving coefficient, relative to that of nHRP. The apparent charge selectivity was reduced, but not eliminated, when radiolabels were employed (76, 77). The ratio of nHRP to aHRP sieving coefficients was reduced to two to three compared with a value of eight to nine in the original report (84).

We turn now to more recent studies by Haraldsson and co-workers, which provide additional evidence in favor of glomerular charge selectivity. Using the IPK preparation at 8°C to inhibit tubular activity, Ohlson et al. (73) found the fractional clearances of albumin and Ficoll of comparable size ($r_s = 36 \text{ Å}$) to be 0.0019 and 0.021, respectively. Using the cooled IPK preparation to examine the filtration of somewhat larger proteins ($r_s = 40–42 \text{ Å}$), Lindstrom et al. (64) showed that the fractional clearance of anionic lactate dehydrogenase (LDH) was less than that of a slightly cationic isoform.

In a comparison of the forms of HRP and LDH with differing charge, variations in molecular shape are not an issue. However, Ficoll is spherical and albumin is modeled more accurately as a prolate spheroid with an axial ratio of ~3.3 (2, 48, 60, 99). To what extent would that difference in molecular shape account for the 10-fold difference in sieving coefficients between Ficoll and albumin? The link between membrane partitioning and sieving (e.g., Eq. 7) suggests that a partial answer would be provided by the theoretical effect of molecular shape on $\Phi$ in a random-fiber matrix such as that used to represent GBM. Applying a recent excluded volume theory (60) to the parameter values in Table 2, the results were $\Phi = 0.0234$ for BSA and $\Phi = 0.0219$ for Ficoll. This difference is not only very small but is in the wrong direction to contribute to the low sieving coefficient for albumin. Supporting the conclusion that the nonspherical shape of albumin is of minor importance are data for $\Phi K_d$ and $\Phi K_c$ in agarose gels of varying concentration, which show little difference between the results for Ficoll and various globular proteins, including BSA (50, 53).

Another recent finding with the cooled IPK is that reductions in the ionic strength of the perfusate decreased the fractional clearances of both aHRP and albumin, without affecting those of Ficoll (96). Because low ionic strengths amplify electrostatic interactions by reducing Debye screening, this was taken as evidence for functional, fixed negative charges. However, with experiments with isolated GBM at reduced ionic strength (9), this shows only that charge was influential at the lower ionic strength. Because charge interactions will tend to be fully suppressed above a certain ionic strength (i.e., when the Debye length is very small relative to the spaces accessible to permeating macromolecules), examining normal and reduced ionic strengths does not exclude the possibility that the charges are fully screened under normal conditions.

The significance of the cellular processing of DS depends on where the uptake occurs and the time required for intracellular levels to become constant. The half-time for accumulation of label in the glomeruli of isolated perfused kidneys (IPK) was <5 min (100), indicating that for clearance measurements done over much longer periods, time-dependent accumulation in the glomerulus will be unimportant. This is true for the studies by Mayer et al. (68) and Guasch et al. (41), where bolus doses of $^3\text{H}$-DS were followed by constant infusions, and sample collections were not begun until after 45–60 min of equilibration. Thus the rate at which the tracer crossed the glomerular barrier in those studies should have equaled its rate of appearance in urine, as assumed in the fractional clearance methodology. Under such steady-state conditions, if the cellular uptake and desulfation were downstream of the barrier (i.e., by epithelial cells from Bowman’s space fluid), total tritium in urine (reflecting both intact and desulfated DS) would accurately reflect filtration of anionic DS and no new interpretation would be needed. Other possibilities include uptake by the foot processes from the filtration slits or GBM and uptake by the endothelial cells from the GBM or plasma. Potentially most significant is endothelial uptake of DS from plasma. If uptake by glomerular endothelial cells were rapid enough to compete with movement through the fenestrae, and if desulfation and release on the contraluminal side of the cells were slow, then entry of DS into the GBM would be slowed by the cellular processing. This would have the effect of reducing the fractional clearance of DS relative to uncharged dextrans.

In support of the concept of DS processing in glomerular endothelial cells, Vyas et al. (105) cited evidence for endothelial endocytosis of sulfated polysaccharides in other organs. Moreover, after an intravenous bolus of $^3\text{H}$-DS in rats, some 78% of the label remaining in plasma was found by affinity chromatography to be desulfated within 2 h (22). However, the evidence tends to be ambiguous for uptake by glomerular endothelial cells specifically. Processing of DS by those cells was not rapid enough to allow detection of an increase in desulfated DS in perfusate collected from the IPK (22). The finding that DS isolated from glomerular digests (100) or vesicles (105) has a similar size distribution to that in plasma could mean that it is of endothelial origin, as argued, or that size-based fractionation occurs mainly at the level of the epithelium. Similarly, the finding of similar amounts of DS in vesicles isolated from filtering and nonfiltering perfused kidneys (105) is consistent with either cellular source. That is, the very small values of $\text{Pe}$ estimated for the GBM (as discussed in Experimental Assessment of GBM and Cellular Contributions) imply that diffusion is rapid enough that water filtration will not greatly speed access of macromolecules to the epithelial cells.
more definite conclusion would be reached by showing that an ionic strength above the physiological elevates the fractional clearances of aHRP and albumin, making them more like those of a neutral test solute such as Ficoll.

A crucial aspect of the controversy over charge selectivity is the manner and extent to which the glomerular barrier restricts the passage of albumin. Two very different hypotheses have emerged. The conventional view, recapitulated recently in Ohlson et al. (73, 74), is that the sieving coefficient for albumin is normally quite low, on the order of $10^{-4}$ to $10^{-3}$, due in part to electrostatic interactions between albumin and fixed negative charges in the glomerular capillary wall. An alternative hypothesis proposed in Osicka et al. (78) is that the sieving coefficient of albumin is unaffected by charge and roughly 100-fold higher; using various drugs (including NH$_4$Cl) to inhibit tubular protein reabsorption in the IPK at 37°C, they inferred an albumin sieving coefficient of 0.07. This high sieving coefficient was reconciled with the low concentrations of albumin normally found in proximal tubule fluid by postulating a high-capacity absorption pathway that returns intact albumin from tubular fluid to plasma (78).

A critique of the alternative hypothesis for albumin handling is given in Ohlson et al. (73), who measured fractional clearances of albumin and Ficoll in IPK preparations at both 8 and 37°C. Using NH$_4$Cl at 37°C, they too found a high fractional clearance for albumin (0.02), approaching that for similarly sized Ficoll under those conditions (0.04). They argued that the apparent loss of barrier selectivity for albumin in the IPK at 37°C, and especially the loss of charge selectivity, is the result of irreversible glomerular injury due both to hypoxia-reperfusion and to drugs used to inhibit tubule function. They also criticized the concept of rapid reabsorption of intact albumin, citing inconsistencies with the finding of Maunsbach (67) that practically all albumin is degraded during reabsorption. Finally, they noted the electron microscopic evidence of Ryan and Karnovsky (91) that albumin is efficiently excluded from the glomerular capillary wall, and micropuncture measurements by Tojo and Hitoshi (101), which confirm that albumin concentrations in early proximal tubule fluid are very low. This last study is noteworthy in that a technique was devised to avoid the difficult problem of sample contamination with subcapsular fluid; the sieving coefficient estimated for albumin was $6 \times 10^{-4}$ (101). We find all of these arguments persuasive.

To summarize our conclusions from the various experimental studies, the concept that charge selectivity contributes to the exclusion of albumin and other polyanions from glomerular filtrate remains viable, despite technical concerns. It is certain that DS is not as inert a tracer as once believed, and it is likely that earlier studies (e.g., with DS and aHRP) overestimated the effects of charge. Indeed, a major lesson has been how difficult it is to design experiments to test charge selectivity in vivo. Nonetheless, recent results with the IPK tend to reinforce, rather than negate, the conclusions from earlier fractional clearance studies in vivo.

**Charge Selectivity Models**

Structure-based models for the glomerular filtration of charged macromolecules, comparable to those discussed above for water and uncharged solutes, have not yet been developed. An early model for glomerular charge selectivity was based on the concept of Donnan exclusion from a homogeneous, charged membrane (29). Although able to describe certain experimental trends, that model has three aspects that are unrealistic. First, the Donnan approach treats permeating ions as if they were point charges, so that molecular size and shape are not accounted for in calculating the electrostatic interactions. Second, the Donnan model regards the fixed charge as being uniformly distributed within a fluid volume, rather than being localized on the surfaces of macromolecular fibers or cells. Third, the model does not distinguish one part of the capillary wall from another. For these reasons, the effective concentration of fixed, negative charges ($C_m$) derived by applying the Donnan model to fractional clearance data must be viewed with great caution. As noted previously (41), such values of $C_m$ are valid only for limited comparative purposes, as when the same test molecule is used to assess differences in the glomerular capillary wall between two experimental conditions. The same value of $C_m$ may not apply to other test molecules, due to size and/or shape differences (41, 66), and it is unlikely to correspond to a charge density of any part of the capillary wall determined by chemical assay.

The limitations of the Donnan model merit reemphasis because the failure of $C_m$ to correspond to a realistic charge density has been used as an argument against the possibility of glomerular charge selectivity (20, 21, 109). That argument is logically flawed, as may be seen by analogy. That is, pore models have been successfully used to correlate data on glomerular size selectivity, often yielding effective pore radii of $\sim 50 \text{ Å}$ (66), but there is no microscopic or compositional basis for the existence of straight, cylindrical pores of that size passing through the capillary wall. Arguing against charge selectivity on the basis of $C_m$ values is akin to arguing against size selectivity on the basis of there being no anatomic correlates of pores. In other words, the microstructural and/or nanostructural limitations of the models, as applied to the glomerular capillary wall, do not invalidate the physiological data.

Ohlson et al. (74) recently presented a one-dimensional model (i.e., without cell coverage effects) in which purely charge-selective and size-selective barriers were placed in series. The Donnan approach was used to describe charge effects in the upstream part of the barrier (identified with the endothelial glycocalyx), and pore theory was used to model size selectivity in the downstream part (GBM and/or filtration slits). By exploring the idea that charge and size selectivity reside largely in different parts of the capillary wall,
this work avoids one of the limitations of the early study noted above. A model has been developed to describe electrostatic effects on the partitioning of spherical macromolecules in random arrays of charged fibers (50). Because it accounts for molecular size and the localization of the fixed charges on fiber surfaces, this theory (or extensions of it) offer the prospect of overcoming the other limitations of the early model and of the Donnan model for the glycocalyx. Finally, by calling attention to what might be a crucial contribution of the endothelial glycocalyx, the work of Ohlson et al. (74) suggests that its role in water filtration and filtration of neutral macromolecules might also merit reconsideration.

Effects of Proteins on Sieving of Tracers

As already noted, Ficoll and Ficoll sulfate sieving coefficients measured in isolated GBM were found to be indistinguishable, at any given value of \( r_s \) (9). However, the same study revealed a pronounced upward shift in the sieving curves of either tracer when BSA was present in the retentate at a concentration of 4 g/dl. (Due to concentration polarization in the stirred ultrafiltration cell, the BSA concentration at the upstream membrane surface was calculated to be higher, 6.2 g/dl.) The results for Ficoll, with and without BSA, are shown in Fig. 5. Because the hydraulic permeability of the GBM filters was unaffected by BSA, the shift in the sieving curves apparently was not due to an alteration of the intrinsic properties of the GBM (i.e., a result of binding of BSA to the membrane). An increase in \( \Theta \) would result from the measured reduction in filtrate velocity in the presence of BSA, caused by its osmotic pressure; this is the Pécellet number effect discussed in connection with Eq. 7. However, as shown by the theoretical curve in Fig. 5 labeled “osmotic only,” this was calculated to account for only about one-third of the increase, on average. It was suggested that the remainder of the BSA effect might be due to another physical phenomenon, namely, a tendency of steric interactions with BSA to facilitate entry of the tracers into the membrane (9). That phenomenon had been the subject of several previous theoretical and experimental investigations, which showed that the equilibrium partition coefficient of a macromolecule between a bulk solution and a porous or fibrous material is dependent on its concentration (3, 10, 38, 40). In essence, steric interactions among molecules in a concentrated solution cause entry into the porous or fibrous material to be more favorable thermodynamically than if the solution were diluted. As shown in Eq. 7, increases in \( \Phi \) will tend to increase \( \Theta \). Thus the sieving coefficients of Ficoll and BSA in synthetic membranes were found to increase with increasing solute concentration (70).

The hypothesis that BSA, as an abundant solute, could affect the partitioning and sieving of a tracer (Ficoll or Ficoll sulfate) has been examined recently in detail. This was done by first extending the theory for partitioning in random fiber arrays, which had been limited to dilute (71) or concentrated (38) solutions of a single, spherical solute, to include interactions among unlike macromolecular solutes (60). The theory for the sieving of macromolecular solutes was then extended to allow for different values of \( \Phi \) at the upstream and downstream surfaces of a membrane, which would be a consequence of having different concentrations of a second, abundant solute (61). As shown by the curve labeled “osmotic and partitioning” in Fig. 5, it was found that the predicted effect of BSA on Ficoll partitioning was more than sufficient to account for the remainder part of the upward shift in the Ficoll sieving curve. At physiological concentrations, predictions for tracer sieving in the presence of BSA were found to be insensitive to the assumed shape of the protein (sphere or prolate spheroid). For protein mixtures, the theoretical effect of 6 g/dl BSA on the partitioning of spherical tracers was indistinguishable from that of 3 g/dl BSA and 3 g/dl IgG. This effect of abundant proteins on the partitioning of tracers has not yet been fully incorporated into simulations of macromolecule filtration in vivo. Lacking the basis for a more precise description, the model of Edwards et al. (35) assumed that proteins would increase \( \Phi \) of each tracer molecule by a constant factor.

Ohlson et al. (74) recently reported Ficoll sieving data in the IPK (at 8 °C) in the presence of either 1.8 or 5.0 g/dl albumin. The fractional clearances over much of the size range examined were significantly elevated at the higher protein concentration, qualitatively consistent with the effects described above.

Proteins may also have more specific effects on glomerular permeability. Orosomucoid is a serum protein that is thought to have a role in determining capillary permeability by maintaining and reinforcing the charge barrier (24, 43). Haraldsson et al. (42) and Johnsson and Haraldsson (51) demonstrated that orosomucoid influences the glomerular barrier by showing that the clearance of albumin in the IPK was four to five times lower when orosomucoid was present.

GBM Nanostructure and Macromolecule Filtration

As with \( \kappa \), efforts to predict the values of \( \Phi K_s \) and \( \Phi K_a \) in the GBM have been based largely on representing it as an array of randomly oriented fibers with fluid-filled interstices. Before considering the GBM specifically, we first survey the various theoretical results that might be used for this purpose. For media containing fibers of uniform size, theories for the partitioning of neutral macromolecules been developed for dilute (71) or concentrated (38) solutions of rigid spheres, for dilute or concentrated mixtures of rigid solutes of arbitrary shape (60), and for dilute solutions of random-coil chains (108). For neutral, rigid solutes, mixtures of fiber sizes have also been considered (60). Hydrodynamic models to predict \( K_s \) in fiber matrices have been described in Johnson et al. (49), Clague and Phillips (17), and Phillips (81), but there is a paucity of information on \( K_a \). The convective reflection coefficient \( (\sigma) \) for a random fiber matrix is related to \( K_a \) as \( \sigma = 1 - \Phi K_a \), and it has been assumed that \( \sigma = (1 - \Phi)^2 \) (23), a
relationship derived originally for the osmotic reflection coefficient in cylindrical pores (4). This prediction for \( \sigma \) (or \( \Phi K_c \)) has been found to be unreliable for proteins in polyacrylamide gels (56) and for proteins or Ficoll in agarose gels (52, 53). The same is true for an early diffusion model (72), frequently quoted in the literature, which has been reported to greatly overestimate \( K_0 \) in agarose gels (49). In contrast, the theories for partitioning and the recent theories for diffusion appear to be reasonably accurate (47, 49, 50, 81).

In an effort to model the sieving results for Ficoll (without BSA) shown in Fig. 5, Bolton and Deen (8) represented the GMB as an array of fibers of uniform radius. They evaluated \( \Phi K_c \) using the theory of Ogston (71) for \( \Phi \) and that of Johnson et al. (49) for \( K_d \) but chose to employ an empirical expression for \( \Phi K_c \) similar to Eq. 10. It was found that a fiber-matrix model based on a single population of fibers could accurately predict both the sieving curve for Ficoll and the value of \( \kappa \), but only if the volume fraction of fibers was assumed to be unrealistically large. It was concluded that fiber-matrix models based on a uniform fiber size do not adequately relate the microstructure of the GMB to its permeability properties. The success of the two-fiber model in describing partitioning of dextrans and serum albumin in porous glass.

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