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Topical Review

Biophysical Basis of Glomerular Permselectivity

William M. Deen, Charles R. Bridges, and Barry M. Brenner

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, and Laboratory of Kidney and Electrolyte Physiology and Departments of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

Summary. The mammalian glomerular capillary wall normally restricts the transmural passage of plasma proteins while offering little resistance to the filtration of water and small solutes. The basis for this selectivity has been explored extensively in recent years, through clearance measurements of endogenous (mainly albumin, transferrin, and immunoglobulins) and exogenous (horseradish peroxidase) proteins, and a variety of nonprotein polymers such as dextrans and polyvinylpyrrolidone. In conjunction with efforts to localize particulate and soluble tracers by high resolution ultrastructural techniques, such measurements have now made it possible to define the determinants of the glomerular filtration of macromolecules in terms of discrete structural barriers as well as such biophysical influences as hemodynamics and the molecular size- and charge-selective characteristics of the capillary wall.

These experimental approaches have been aided greatly by the development of theoretical models that enable investigators to describe macromolecular filtration in terms of hydrodynamic principles applied to isoporous membranes. Although the initial models failed to consider the important role of membrane fixed negative-charge characteristics in influencing protein filtration, this shortcoming has led to the recent introduction of a theoretical model that also takes this factor into consideration. The aim of this brief review is to summarize these various theoretical approaches to the understanding of glomerular permselectivity and, wherever possible, to cite specific tests of these theories based on experimental studies in humans and animals.

Key Words glomerular permeability · glomerular filtration · proteinuria · transport of macromolecules · renal microcirculation · membranes, hindered transport in pores

Effects of Molecular Properties on Glomerular Selectivity

The most useful quantitative method for studying glomerular capillary permeability to macromolecules has been the measurement of fractional clearances. The fractional clearance of some test macromolecule, T, is defined as the urinary clearance of T divided by the glomerular filtration rate of water (GFR). If the clearance of inulin is used to measure GFR, the fractional clearance is given by the urine-to-plasma concentration ratio for the test macomolecule, divided by the corresponding concentration ratio for inulin. If, like inulin, the test macromolecule is not reabsorbed or secreted, the fractional clearance of T will exactly equal the Bowman's space-to-plasma concentration ratio of T. This ratio is denoted by θ:

$$\theta = \frac{C_{TB}}{C_{TA}} \tag{1}$$

where C_{TB} is the average concentration of T in Bowman's space and C_{TA} is the concentration of T in afferent arteriolar (or systemic) plasma. Thus, with the choice of suitable test macromolecules, only urine and plasma samples are required in order to calculate θ.

The size-selectivity of the glomerular capillary wall has been demonstrated effectively by using certain series of uncharged, exogenous polymers covering a range of molecular sizes. This allows size effects to be examined without complications due to differences in molecular charge or chemical structure. Dextran, a polymer of D-glucopyranose, and polyvinylpyrrolidone (PVP) both fulfill the requirement of ideal test macromolecules, in that they are not appreciably reabsorbed or secreted by the renal tubule and are nontoxic in tracer quantities. An example of fractional clearance data obtained using neutral (uncharged) dextran is shown in Fig. 1. The fractional clearance of dextran is shown as a function of its effective molecular radius, as reported by Bohrer et al. [8] for the normal hydropenic rat. The measurement of the molecular radius of dextran is based on its elution from gel chromatographic columns calibrated with several proteins of known Stokes-Einstein radius [17], and provides a better basis for comparing the filtration of macromolecules of differing structure than does molecular weight. A value of 1 on the ordinate of Fig. 1 indicates no measurable restriction to filtration, which is the

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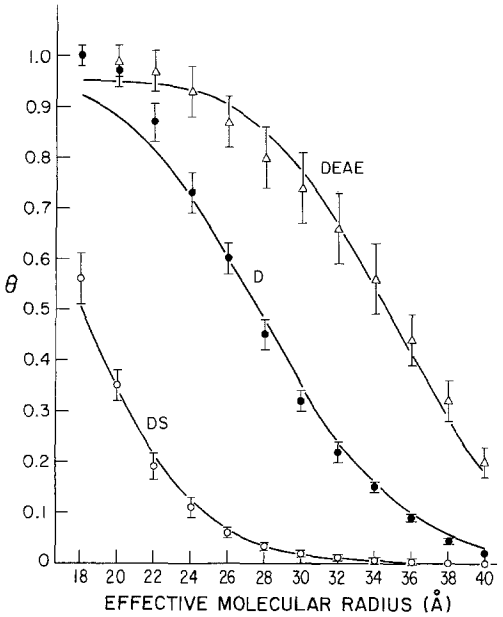

Fig. 1. Filtrate-to-plasma concentration ratio (θ) as a function of molecular size for tritiated dextran sulfate (*DS*), neutral dextran (*D*), and diethylaminoethyl dextran (*DEAE*). Points represent mean values ± 1 SE measured by Bohrer et al. [6, 8] in the normal Munich-Wistar rat. The solid curves were calculated theoretically using the membrane parameters: $K_f = 4.8$ nl/(min·mm Hg), $r_p = 47$ Å, and $C_m = 165$ meq/liter. (Reproduced from Deen, Satvat, and Jamieson, *Am. J. Physiol.* **238**:*F*126, 1980, with permission.)

case for neutral dextrans with effective radii less than about 18 Å. Above this radius dextran fractional clearance decreases progressively with increasing size, approaching zero for molecular radii greater than about 42 Å. Fractional clearance data similar to those shown in Fig. 1 for neutral dextran in the rat have also been reported in other experimental animals and in humans [2, 12, 27–29, 32, 40, 45, 47].

The finding that θ for serum albumin is ~ 0.001 or less, as inferred from micropuncture measurements of albumin concentration in Bowman's space or early proximal tubule fluid in the rat [25, 26, 33, 36], indicates that glomerular filtration of macromolecules is not governed solely by molecular size. As indicated by the solid circles in Fig. 1, the fractional clearance of neutral dextran of the same molecular radius, 36 Å, averages ~ 0.1, some two orders of magnitude greater than that for albumin. However, such a comparison does not lead to very specific conclusions about the glomerular barrier, because neutral dextran and albumin differ in at least two respects, molecular charge and molecular shape or configuration. Neutral dextran is an uncharged, nearly linear polymer expected to behave as a flexible coil in solution, whereas albumin is a polyanion in physiological solution and has a relatively rigid, globular configuration.

To provide a specific test of the effects of molecular charge, Chang et al. [14] examined the transglomerular transport of dextran sulfate, an anionic polymer structurally similar to neutral dextran. At any given molecular radius, fractional clearances of dextran sulfate in the rat were found to be lower than those for neutral dextran [14]. Similar results with dextran sulfate, obtained later by Bohrer et al. [8] are shown by the open circles in Fig. 1. This observed restriction to the filtration of polyanions, relative to neutral macromolecules, is believed to be the result of electrostatic interactions with fixed, negatively charged components of the glomerular capillary wall. Consistent with this interpretation is the finding of enhanced filtration of polycations. Rennke et al. [41–43] found that cationic forms of ferritin and horseradish peroxidase were transported across the glomerular capillary wall of the normal mouse and rat to an extent considerably greater than were their neutral or anionic counterparts. Similarily, Bohrer et al. [6] demonstrated later that the fractional clearances of diethylaminoethyl (DEAE) dextran, a cationic derivative of dextran, exceeded those for neutral dextran over a wide range of molecular radii. These results for DEAE dextran are also illustrated in Fig. 1 (open triangles)[1].

The functional importance of electrostatic interactions between the glomerular capillary wall and circulating macromolecules is further emphasized by the results of studies on the pathogenesis of proteinuria. In rats with a mild form of nephrotoxic serum nephritis (NSN), Chang et al. [15] found fractional clearances of neutral dextrans to be reduced below the values for normal controls, while Bennett et al. [3] showed that those for dextran sulfate were increased. These findings suggest that proteinuria in this disorder is a specific consequence of the reduction in fixed negative

[1] In general, linear polyelectrolytes are expected to have more rigid and elongated structures than the corresponding neutral polymers, because the conformations of the polyelectrolytes are restricted by intramolecular electrostatic repulsions. Accordingly, the average three-dimensional configurations of dextran sulfate and DEAE dextran may be similar to one another, but somewhat different from that of neutral dextran. To the extent that these configurational differences were important, they would be expected to cause fractional clearances of dextran sulfate and DEAE dextran to deviate in the same direction from that of neutral dextran of similar radius. The finding that dextran sulfate fractional clearance is reduced while that of DEAE dextran is enhanced, as shown in Fig. 1, indicates that in this case configurational differences are less important than electrostatic interactions between the polyelectrolytes and the glomerular capillary wall.

charges on the diseased glomerular capillary wall, leading to less restriction of the filtration of circulating polyanions such as albumin and dextran sulfate. In support of this hypothesis, the glomerular fixed charge content has been shown using histochemical techniques to be diminished in a variety of glomerulopathies associated with proteinuria [4, 5, 13, 18, 34, 35, 37], a finding also confirmed in NSN rats in the study by Bennett et al. [3].

A second experimental model of proteinuria, studied by Bohrer et al. [7], was induced by the administration of puromycin aminonucleoside (PAN) to rats. Fractional clearances of neutral dextran were decreased, in accord with the findings of Buerkert et al. [11], who reported reductions in the transglomerular passage of neutral PVP covering a wide range of molecular sizes in PAN-treated rats. Fractional clearances of dextran sulfate, however, were shown by Bohrer et al. [7] to be increased in PAN rats, relative to normal controls. Thus, the findings were quite similar to those in rats with NSN. The enhanced fractional clearances of dextran sulfate, in the absence of similar changes with neutral dextran, suggest that PAN reduces the electrostatic barrier to circulating polyanions, as does NSN. In accord with this explanation, several studies have shown that the binding of cationic "stains" to anionic sites on the glomerular capillary wall is reduced appreciably in aminonucleoside-treated rats, as compared to controls [4, 5, 13, 35, 37].

In addition to molecular size and charge, molecular configuration also affects the selectivity of the glomerular capillary wall. Bohrer et al. [9] demonstrated such an effect by comparing fractional clearances in the rat of neutral dextran and ficoll, an uncharged, crosslinked copolymer of sucrose and epichlorohydrin. Analysis of data on sedimentation rates, intrinsic viscosities, and specific volumes of these polymers showed that ficoll could be more nearly represented as a rigid sphere than could dextran [9]. At any given molecular radius, ficoll was filtered less readily than dextran, the fractional clearance of ficoll at a molecular radius of 36 Å being about one-half that of dextran. In another demonstration of the effects of molecular configuration, Rennke and Venkatachalam [44] found θ for native horseradish peroxidase (HRP) in the rat to be approximately one-seventh that of neutral dextran of comparable radius (28 Å). This enzyme has an isoelectric point within the physiologic pH range [44], and does not migrate appreciably in gel electrophoresis at pH 7.4 [20], so that it may be assumed to be essentially uncharged. Accordingly, the lower value of θ for HRP than for dextran is attributable to differences in molecular configuration.

It is apparent from these studies that in comparing values of θ for different macromolecules, effects of molecular size, charge, and configuration should all be considered. As discussed in the next section, reasonably successful theories have been developed to describe size and charge effects; little progress has yet been made, however, in the quantitative treatment of molecular configuration as it relates to glomerular selectivity.

Theory of Glomerular Size and Charge Selectivity

The Bowman's space concentration for a given macromolecule is a measure of the transcapillary flux of that solute, relative to the transcapillary volume flux. The Bowman's space-to-plasma concentration ratio for macromolecule T is related to these fluxes by

$$\theta = \frac{\langle J_T \rangle}{C_{TA} \langle J_V \rangle} = \frac{\langle J_T \rangle S}{C_{TA}\,\mathrm{SNGFR}} \tag{2}$$

where $\langle J_T \rangle$ and $\langle J_V \rangle$ are solute and volume fluxes, respectively, averaged over the glomerular capillary network. The second equality in Eq. (2) is obtained by noting that the single nephron GFR (SNGFR) is the product of $\langle J_V \rangle$ and the capillary surface area per glomerulus, S.

In relating θ to intrinsic properties of the capillary wall and test macromolecule, we consider first the quantities that determine SNGFR. The local volume flux at any point along a glomerular capillary is given by [21]

$$J_V = k(\Delta P - \pi_G) \tag{3}$$

where k is the effective hydraulic permeability of the capillary wall and ΔP is the transmural hydraulic pressure difference. π_G is the intracapillary colloid osmotic pressure, which may be calculated from total plasma protein concentration at any point along a capillary [21]. Under normal circumstances the colloid osmotic pressure in Bowman's space is negligible. The axial pressure drop along a glomerular capillary due to blood flow has been inferred to be quite small [10, 31], and Bowman's space pressure is expected to be nearly independent of position along a glomerular capillary. Formation of the nearly protein-free ultrafiltrate, however, causes π_G to rise from a minimum value at the afferent end of the capillary (corresponding to systemic arterial plasma) to a value by the efferent end that equals ΔP, at least in the normal hydro-

penic rat and monkey [10]. Thus, J_V normally declines from a maximum value at the afferent end of the capillary to zero by the efferent end, where filtration pressure equilibrium has been achieved.

Mathematical models developed to relate local volume flux (J_V) to SNGFR have generally assumed steady flow through a number of identical glomerular capillaries in parallel, each having uniform values of k and circumference [21, 30]. Conservation of total mass and of plasma proteins leads to the relations [21]

$$\frac{dQ}{dy} = -J_V S \tag{4}$$

$$\frac{d(Q C_P)}{dy} = 0 \tag{5}$$

where Q is glomerular plasma flow rate, C_P is total protein concentration, and y is the normalized position coordinate along an idealized capillary (i.e., $y=0$ at the afferent end and $y=1$ at the efferent end). Equation (4) relates the change in plasma flow rate to the transcapillary volume flux, while Eq. (5) expresses the assumption of negligible filtration of plasma proteins. Integrations of Eqs. (4) and (5), using Eq. (3) and a relation between π_G and C_P, establishes the relationship between capillary hydraulic permeability and SNGFR. It is apparent from Eq. (4) that the significant permeability parameter for water is $K_f = k S$, the glomerular ultrafiltration coefficient. K_f is an intrinsic property of the glomerular capillary network and has been determined directly from micropuncture measurements of glomerular pressures and flow rates in the Munich-Wistar strain of rats, under a variety of physiological and pathophysiological conditions [10].

The most useful theoretical descriptions for transport of macromolecules across the glomerular capillary wall have been based on the concept of hindered movement of solutes through water-filled pores having molecular dimensions. Such models, first applied to capillaries by Pappenheimer et al. [38, 39], usually envision the capillary wall as containing numerous identical, cylindrical pores. Macromolecular solutes are generally treated as solid spheres, and the solvent and low-molecular-weight solutes are regarded as a continuum fluid. Within this theoretical framework, two distinct mechanisms act to reduce macromolecule fluxes below the values that would be seen in very large pores. The first of these is a partitioning phenomenon, whereby the macromolecule, by virtue of its size, shape (for nonspherical molecules), or molecular charge is partially excluded from the pore. For uncharged, spherical molecules this partitioning effect can be deduced from simple geometric arguments based on the idea that the center of the solute sphere does not have access to a region within one solute radius of the pore wall. The ratio of the average concentration in the pore to that in the bulk solution outside is then given by $\phi = (1-\lambda)^2$, where λ is the ratio of solute radius to pore radius [1]. For nonspherical or charged solutes, additional effects come into play and simple analytical expressions for ϕ are no longer obtained [1, 19, 46]. The net result, generally, is that even at equilibrium, the average concentration of a given macromolecule within a pore is less than that in the adjacent bulk solution. The second mechanism for the reduction of macromolecule fluxes in small pores is based on the hydrodynamic retardation of solute movement due to the nearby presence of the pore wall [1]. For solutes modeled as uncharged solid spheres, these hydrodynamic effects once again depend only on the relative solute size, λ. For a more complete discussion of theories of partitioning and hindered particle motion in the context of glomerular filtration, the reader is referred to a recent review by Deen et al. [19].

On the basis of the concepts discussed above, the flux of an uncharged macromolecular solute across the glomerular capillary wall can be expressed as [22]:

$$J_T = \frac{J_V C_T K_C \phi}{1 - \exp(-\alpha)(1 - K_C \phi)} \tag{6}$$

$$\alpha = \frac{K_C J_V \delta}{f K_D D_T}. \tag{7}$$

Equation (6) relates the local solute flux (in mass or moles per unit time per unit area) at any point along a capillary to the volume flux and the concentration of solute T in capillary plasma (C_T). The dimensionless group α is a measure of the relative importance of diffusion and convection to solute transport [19], and contains the fraction (f) of the capillary surface area occupied by pores, the pore length (δ), and the diffusivity of T in bulk solution (D_T). The steric partitioning coefficient, ϕ, has already been defined, and K_C and K_D are hydrodynamic factors which account for hindrances to convective and diffusive movement of solute[2], respectively. All three of these size-dependent quantities (ϕ, K_C, K_D) approach unity for

[2] The notation used here for the hindrance factors follows that of Deen, Satvat, and Jamieson [22]. In terms of the quantities H and W defined by Deen, Bohrer, and Brenner [19], $H = \phi K_D$ and $W = \phi K_C$.

small solutes ($\lambda \to 0$) and approach zero for large solutes ($\lambda \to 1$).

For transport through identical cylindrical pores, the ultrafiltration coefficient (K_f) can be related to quantities appearing in Eqs. (6) and (7) using the Poiseuille equation for flow through cylindrical tubes. The result is:

$$K_f = \frac{fS}{\delta} \frac{r_p^2}{8\eta} \tag{8}$$

where r_p is the apparent pore radius and η is the viscosity of the electrolyte solution in the pore. It is apparent from Eq. (8) that if K_f and r_p are known, the quantity fS/δ is automatically determined and should not be viewed as an independent property of the glomerular barrier. The parameter α may then be written as

$$\alpha = \frac{K_C(\Delta P - \pi_G)}{K_D D_T} \frac{r_p^2}{8\eta}. \tag{9}$$

It can now be seen that according to this theory, the rate of filtration of uncharged solute T depends on only two membrane properties, K_f, and r_p. Accordingly, if K_f is evaluated from SNGFR and its determinants [10, 21], r_p may be estimated from the filtration rate of any uncharged macromolecule of specified size. As with J_V and SNGFR, a mass balance equation is required to relate the local flux of the macromolecule (J_T) to a measurable quantity (either θ or single glomerular clearance, $\theta \cdot$SNGFR). This relation, analogous to Eqs. (4) and (5), is

$$\frac{d(Q C_T)}{dy} = -J_T S. \tag{10}$$

Integration of Eq. (10) (using Eqs. (3), (6) and (9)) and averaging of the resulting fluxes along the capillary length allows calculation of θ. Values of θ calculated in this way can be matched to observed values by adjusting r_p.

Effects of electrical charge have been incorporated into the solute flux expression by assuming that the glomerular capillary wall can be represented as a barrier containing a uniform concentration of fixed negative charges, C_m [22]. According to this model, the fixed charges cause the intramembrane potential (within the capillary wall) to be negative with respect to that in plasma or Bowman's space, a result of Donnan equilibria established at the respective membrane-solution interfaces. Partitioning of charged solutes between the membrane and external solutions is then influenced not only by molecular size but also by these potential differences. Let $\Delta\psi(0)$ and $\Delta\psi(\delta)$ represent potentials in the external solution (0 for plasma and δ for Bowman's space) minus those within the membrane. These are most conveniently expressed as dimensionless potentials, referenced to the product of the gas constant and absolute temperature divided by Faraday's constant (26.7 mV at 37 °C), and are positive numbers. Both depend primarily on C_m, increasing as C_m increases; $\Delta\psi(0)$ is influenced to a lesser extent by the total protein concentration in the capillary lumen (C_P), decreasing as C_P increases. The pore-to-bulk solution concentration ratio, given by $\phi(\lambda)$ for neutral solutes, is assumed in this model to equal $\phi \exp(Z_T \Delta\psi)$ for charged macromolecules. Z_T is the molecular charge, and different values of $\Delta\psi$ are used at the two sides of the membrane, as indicated above. Details concerning the calculation of electrical potentials for this model, and the influence of electrokinetic phenomena on glomerular filtration, are discussed elsewhere [22].

The solute flux expression for charged macromolecules, analogous to that given by Eq. (6) for neutral macromolecules, is [22]

$$J_T = \frac{J_V C_T K_C \exp[Z_T \Delta\psi(0)]}{1 - \exp(-\alpha)\{1 - K_C \phi \exp[Z_T \Delta\psi(\delta)]\}}. \tag{11}$$

For relatively large or very negatively charged macromolecules, the term $K_C \phi \exp[Z_T \Delta\psi(\delta)]$ in the denominator of Eq. (11) will become much smaller than unity. The solute flux in this case can be seen to vary approximately as $\exp[Z_T \Delta\psi(0)]$, a very strong dependence on molecular charge. For polyanions ($Z_T < 0$) this exponential factor will be less than unity, and comparison of Eqs. (6) and (11) indicates that J_T will be less than that of an uncharged macromolecule of the same size, since the two are assumed to have the same values of K_C, ϕ and α. Similarly, J_T for a polycation ($Z_T > 0$) will be enhanced in this model. Note that for an uncharged macromolecule ($Z_T = 0$), Eq. (11) reduces exactly to Eq. (6).

The model just discussed predicts that for charged macromolecules there will be a strong dependence of J_T on C_m, since C_m is the primary determinant of $\Delta\psi(0)$. Accordingly, with values of K_f and r_p already determined from glomerular filtration rates of water and neutral macromolecules, values of C_m can be estimated from filtration rates of charged macromolecules. The glomerular barrier is then fully characterized by the three membrane properties, K_f, r_p, and C_m.

It should be noted that there is an inconsistency in the assumption that the glomerular "membrane" has a homogeneous distribution of fixed

charges, yet also possesses discrete pores. In the context of a pore model, the fixed charges would be confined to the pore walls, and electrostatic interactions with permeating macromolecules would be greater near the pore wall than at the pore center. More rigorous methods for calculating electrostatic effects on solute partitioning between pores and bulk solution are becoming available [46], so that a more consistent theoretical treatment should be possible in the near future. Despite its limitations, the present model has nonetheless proved useful in describing both size and charge-selectivity in glomerular filtration in the rat [22, 23].

Glomerular Permeability Parameters in the Rat

Using the theory outlined in the previous section, the size-selectivity of the glomerular capillary wall can be interpreted in terms of effective pore radii. For the neutral dextran data shown in Fig. 1, calculated values of r_p prove to be relatively independent of molecular size, averaging about 47 Å. Presumably, all molecules encounter the same size pores, so that the finding that the "best-fit" value of r_p is independent of molecular size is confirmation that the theory successfully correlates most of the data. Values of θ for neutral dextrans calculated using the theory are shown by the middle solid curve in Fig. 1. It can be seen that a single pore radius of 47 Å provides an excellent representation of the data, except for molecular radii less than about 24 Å, for which the isoporous theory appears to underestimate dextran filtration.

Pore radii obtained in several studies in the rat are shown in Table 1. The first two entries correspond to the same data for neutral dextran as shown in Fig. 1. For other studies using neutral dextran, even in the two forms of glomerular injury examined (NSN and PAN), pore radii are quite similar, falling in the range 45–54Å. Comparable values of r_p, about 50 Å, have also been obtained from fractional clearance data for PVP in dogs [27, 47] and dextran in humans [2, 48]. The lower value of r_p shown in Table 1 for horseradish peroxidase (37 Å) follows from the lower fractional clearance seen for HRP than for dextran of the same gel chromatographic radius, and reflects differences in molecular configuration. For the same reason, effective pore radius for ficoll is calculated to be 5 Å lower than that for dextran, based on fractional clearances measured in the same group of rats [9]. One may conclude that while pore theory correlates effects of molecular size quite well within a given series of macromolecules, values of r_p obtained for one polymer are not necessarily applicable to another.

Table 1. Effective pore radius (r_p) and fixed charge concentration (C_m) for glomerular capillaries in the rat

Condition	Macromolecule	r_p (Å)	C_m (meq/liter)	Ref.
Normal	D, DS	47	160	8
	D, DEAE	47	170	6
	D, DS	54	120	14
	Albumin	(50)[a]	100–130[b]	25, 26 33, 36
	HRP, SHRP	37[b]	60[b]	42
NSN	D, DS, DEAE	54	25	3, 6, 15
PAN	D, DS	45	100	7

Abbreviations used: D, neutral dextran; DS, dextran sulfate; DEAE, diethylaminoethyl dextran; HRP, horseradish peroxidase; SHRP, anionic succinyl derivative of HRP; NSN, nephrotoxic serum nephritis; PAN, puromycin aminonucleoside nephrosis.

[a] Assumed value used in calculation of C_m from albumin data.

[b] There were insufficient data in these studies to calculate the ultrafiltration coefficient. Glomerular pressures and flow rates were assumed to be similar to those reported by other laboratories for normal hydropenic Munich-Wistar rats [10].

Also shown in Table 1 are values of membrane fixed charge density (C_m), obtained from data for various test macromolecules in the rat. For the dextran derivatives, the values of C_m given represent least-squares fits to fractional clearance data covering the entire range of molecular radii studied. For the results in the normal Munich-Wistar rat reported by Bohrer et al. [6, 8], nearly identical values of C_m are obtained from DS and DEAE, 160 and 170 meq/liter, respectively. That a single value of C_m (165 meq/liter) is consistent with data for all molecular sizes of DS and DEAE is shown in Fig. 1, where excellent agreement can be seen between the calculated results (solid curves) and the measured mean values of fractional clearances of both dextran derivatives. As shown in Table 1, a somewhat lower value of C_m (120 meq/liter) is calculated from fractional clearance data reported for DS in an earlier study by Chang et al. [14]. This is a reflection of the somewhat higher fractional clearances for DS reported by Chang et al. [14] than by Bohrer et al. [8]. Whether this discrepancy reflects a difference in the permeability properties of the groups of Munich-Wistar rats studied, a difference in the charge content or other properties of the tracer macromolecules employed, or some other difference between the two studies using DS, cannot be determined.

Much of the experimental literature on the glomerular filtration of proteins is based on urinary clearance measurements, the interpretation of

which is made difficult by the fact that protein reabsorption rates are generally not known precisely, but are substantial. The studies cited in Table 1 using endogenous albumin [25, 26, 33, 36] or native and modified horseradish peroxidases [42] were designed to avoid these difficulties. The range of C_m shown for albumin (100–130 meq/liter) reflects a fivefold variation in the estimates of θ obtained from the various studies, and is remarkably similar to the range of C_m values obtained with charged dextrans. The estimate of C_m obtained from neutral and anionic forms of horseradish peroxidase (60 meq/liter) is considerably lower than the other values for normal rats. A larger value of r_p for the horseradish peroxidases would also yield a larger value of C_m.

Thus, measurements using a variety of test macromolecules in normal rats are consistent with effective fixed-charge densities generally exceeding 100 meq/liter. The studies using dextran derivatives in NSN and PAN yield lower values, 25 and 100 meq/liter, respectively, as shown in Table 1. Although pore radius was virtually unchanged, the ultrafiltration coefficient (K_f) was markedly decreased in both disorders, to value approximately one-third of normal. This reduction in K_f can be attributed almost entirely to a decrease in the parameter fS/δ (Eq. (8)). Thus, in addition to a loss of fixed charge from the glomerular capillary wall in these disorders, there is a reduction in the number of filtering pores (proportional to fS), perhaps accompanied by an increase in pore length (δ).

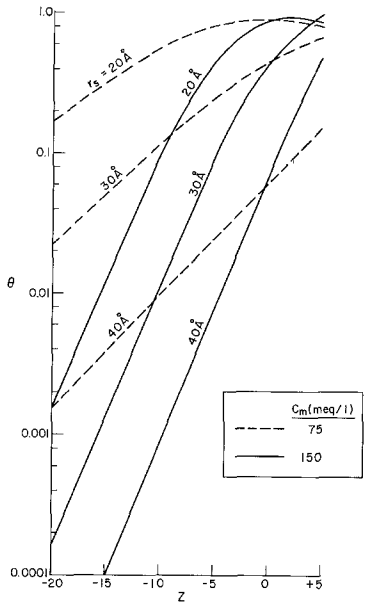

Fig. 2. Filtrate-to-plasma concentration ratio (θ) as a function of molecular charge (Z) and molecular radius (r_s). Results are shown for two values of membrane fixed charge concentration (C_m). Calculations assume $r_p = 50$ Å and other input quantities representative of the normal rat [22]

Determinants of Macromolecule Filtration

Having established values of the membrane parameters that characterize the glomerular capillary wall, it is possible to compare theoretically the effects of various factors on the filtration rates of macromolecules. These factors include molecular properties, membrane properties, and hemodynamic variables such as glomerular plasma flow rate and transmembrane hydraulic pressure difference [23]. Figure 2 illustrates the effects of molecular size, molecular charge, and membrane charge on θ, assuming an effective pore radius of 50 Å and values of K_f and glomerular hemodynamic quantities representative of the normal hydropenic rat [10, 22]. Curves giving θ as a function of molecular charge (Z) are shown for three molecular radii (r_s) at each of two values of membrane fixed charge density (C_m). For uncharged macromolecules ($Z = 0$), θ is shown to decrease by one order of magnitude (a factor of 14) as molecular radius is increased from 20 to 40 Å, irrespective of the value of C_m. Electrical charge is seen to amplify the effect of size for anionic macromolecules ($Z<0$) and diminish it for cationic polymers ($Z>0$). Thus, for polyanions of equal charge such that $Z<-5, \theta$ is predicted to decrease by about two orders of magnitude as r_s increases from 20 to 40 Å. This is true whether C_m values are chosen to represent the normal capillary wall (150 meq/liter) or partial loss of fixed negative charges (75 meq/liter). Of course, within any homologous series of macromolecules, not only r_s but also $|Z|$ will increase with molecular weight.

The linear relationship between $\log\theta$ and Z shown in Fig. 2 for highly anionic macromolecules is a consequence of the exponential dependence of J_T on Z_T shown in Eq. (11), since θ is directly related to the average value of J_T along a capillary (Eq. (2)). The slope of each of the lines in this region can be identified roughly with the average value of the Donnan potential between the capillary lumen and membrane [$\Delta\psi(0)$], which is determined primarily by C_m. Thus, the three curves at $C_m = 150$ meq/liter have slopes similar to one another, and much greater than those for $C_m =$ 75 meq/liter. In other words, for any molecular size, the greater the membrane charge density, the more pronounced is the effect of molecular charge.

It is apparent from Fig. 2 that the combined effects of molecular size and charge can readily

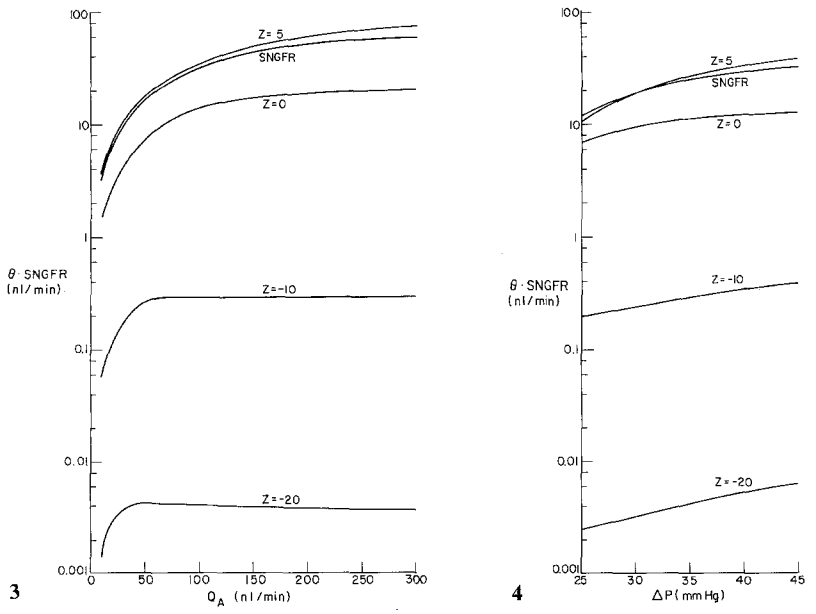

Fig. 3. Single nephron clearance ($\theta \cdot$SNGFR) as a function of glomerular plasma flow rate (Q_A) and molecular charge (Z). Calculations assume $r_s = 30$ Å, $r_p = 50$ Å, $\Delta P = 35$ mm Hg, and other input quantities representative of the normal rat [22]

Fig. 4. Single nephron clearance ($\theta \cdot$SNGFR) as a function of transcapillary hydraulic pressure difference (ΔP) and molecular charge (Z). Calculations assume $Q_A = 75$ nl/min and other input quantities as in Fig. 3

account for variations in θ among different macromolecules covering several orders of magnitude, even when the ratio of solute-to-pore radius is maintained in the range 0.4–0.8. As already noted, experimental evidence in the rat indicates that differences in molecular configuration between uncharged molecules of similar size may also affect θ by as much as one order of magnitude [9, 44]. In considering the pathogenesis of proteinuria, the more pertinent issue is the effect of changes in glomerular membrane properties on θ for a given macromolecule. For a highly anionic protein such as serum albumin ($r_s = 36$ Å, $Z = -19$), effects of membrane charge density (C_m) on θ are likely to be more important than variations in pore radius. For example, decreases in C_m from 150 to 75 meq/liter are calculated to increase θ for albumin by a factor of approximately 100, whereas a doubling of pore radius (from 50 to 100 Å) increases θ by only a factor of 5 [23]. Accordingly, it appears that very extensive amounts of proteinuria may be accounted for by decreases in C_m, irrespective of changes in pore radius.

Hemodynamic perturbations that change SNGFR may also profoundly affect both the fractional and the absolute clearances of macromolecules. Equation (2) shows that unless the absolute clearance of the test macromolecule under consideration, $\langle J_T \rangle S/C_{TA}$, happens to change exactly in proportion to SNGFR, θ will not remain constant when SNGFR is altered. Several theoretical studies have pointed out that in most cases an inverse relationship is to be expected between θ and SNGFR, θ decreasing as SNGFR increases [16, 23, 38, 40, 47]. However, the magnitude and even the direction of the predicted variations in θ depend both on molecular properties and the manner in which SNGFR is changed [16, 23, 24, 47]. In addition to the ultrafiltration coefficient, K_f (a membrane characteristic), SNGFR depends on three "hemodynamic" quantities [10, 21]: initial or afferent glomerular plasma flow rate (Q_A); transcapillary hydraulic pressure difference (ΔP); and afferent plasma protein concentration (C_{PA}). With reference to Eq. (3), increases in Q_A elevate SNGFR by reducing the tendency of protein concentration and oncotic pressure (π_G) to increase in going from the afferent to the efferent end of a capillary, and thereby lowering the average value of π_G opposing filtration [21]. The initial value of π_G is determined by C_{PA}.

θ has been defined such that the absolute clearance of a macromolecule, at the level of a single glomerulus, is given by the product $\theta \cdot$SNGFR. The dependence of absolute clearance on Q_A and ΔP is illustrated in Figs. 3 and 4 for hypothetical macromolecules of 30 Å effective radius and molecular charge ranging from $Z = -20$ to $+5$. Values of SNGFR are also shown for comparison. For the normal hydropenic rat, on which these

calculations are based, $Q_A \simeq 75$ nl/min and $\Delta P \simeq 35$ mm Hg. It can be seen in Figs. 3 and 4 that absolute clearance increases in most cases as SNGFR is elevated, whether this is done by selectively raising Q_A or ΔP. Also, in most cases, absolute clearance increases less than in proportion to the increase in SNFGR, indicating a decrease in θ. The most direct experimental confirmation of the predictions for neutral macromolecules is provided by a study by Chang et al. [17], in which Q_A was elevated to values approximately three times normal by isoncotic plasma volume expansion. For neutral dextrans covering a wide range of sizes, reductions in θ during plasma volume expansion were in close accord with theoretical expectations [17].

For sufficiently anionic or cationic macromolecules, θ and absolute clearance deviate from the main trends just described. Figure 3 shows that absolute clearances for the more anionic macromolecules are relatively insensitive to increases in Q_A, that for $Z=-20$ actually decreasing slightly as Q_A is increased above the normal value of 75 nl/min. This finding is particularly interesting in that it suggests that a mechanism exists for increasing the glomerular filtration rate of water and small solutes without any attendant increase in the filtration of even low molecular weight plasma proteins, provided that they are sufficiently anionic. For sufficiently cationic macromolecules, as indicated for $Z=5$ in Figs. 3 and 4, single nephron clearance may exceed SNGFR ($\theta > 1$) and increase more rapidly than SNGFR as Q_A or ΔP are elevated.

Finally, it should be noted that hemodynamic perturbations can lead to changes in absolute clearance by altering θ in the absence of any effect on SNGFR. For example, reductions in Q_A may be compensated by increases in ΔP to maintain near constancy of SNGFR; this has been found experimentally in the rat during infusion of angiotensin II [8], in which proteinuria is also observed. Theoretical simulations of albumin filtration show that θ may be increased substantially when Q_A is reduced and ΔP simultaneously increased to maintain exact constancy of SNGFR [23]. Under these conditions, θ and absolute clearance for albumin are approximately doubled when Q_A is reduced from 75 to 50 nl/min. If, as suggested by micropuncture studies of albumin filtration and reabsorption in the rat [25, 26, 36], albumin is reabsorbed by a saturable process normally operating near capacity, the percentage increase in urinary albumin excretion could greatly exceed that of filtration. These results indicate that caution is required in attributing proteinuria to glomerular injury, particularly in states in which renal plasma flow rate is decreased and filtration fraction increased, leaving GFR relatively constant.

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References

1. Anderson, J.L., Quinn, J.A. 1974. Restricted transport in small pores. A model for steric exclusion and hindered particle motion. *Biophys. J.* **14**:130–150
2. Arturson, G., Groth, T., Grotte, G. 1971. Human glomerular membrane porosity and filtration pressure: Dextran clearance data analyzed by theoretical models. *Clin. Sci.* **40**:137–158
3. Bennett, C.M., Glassock, R.J., Chang, R.L.S., Deen, W.M., Robertson, C.R., Brenner, B.M. 1976. Permselectivity of the glomerular capillary wall. Studies of experimental glomerulonephritis in the rat using dextran sulfate. *J. Clin. Invest.* **57**:1287–1294
4. Blau, E.B., Haas, D.E. 1973. Glomerular sialic acid and proteinuria in human renal disease. *Lab. Invest.* **28**:477–481
5. Blau, E.B., Michael, A.F. 1972. Rat glomerular glycoprotein composition and metabolism in aminonucleoside nephrosis. *Proc. Soc. Expt. Biol. Med.* **141**:164–172
6. Bohrer, M.P., Baylis, C., Humes, H.D., Glassock, R.J., Robertson, C.R., Brenner, B.M. 1978. Permselectivity of the glomerular capillary wall. Facilitated filtration of circulating polycations. *J. Clin. Invest.* **61**:72–78
7. Bohrer, M.P., Baylis, C., Robertson, C.R., Brenner, B.M. 1977. Mechanism of the puromycin-induced defects in the transglomerular passage of water and macromolecules. *J. Clin. Invest.* **60**:152–161
8. Bohrer, M.P., Deen, W.M., Robertson, C.R., Brenner, B.M. 1977. Mechanism of angiotensin II – induced proteinuria in the rat. *Am. J. Physiol.* **233**:*F*13–*F*21
9. Bohrer, M.P., Deen, W.M., Robertson, C.R., Troy, J.L., Brenner, B.M. 1979. Influence of molecular configuration on the passage of macromolecules across the glomerular capillary wall. *J. Gen. Physiol.* **74**:583–593
10. Brenner, B.M., Baylis, C., Deen, W.M. 1976. Transport of molecules across renal glomerular capillaries. *Physiol. Rev.* **56**:502–534
11. Buerkert, J.E., Mor, J., Murray, B.N., Robson, A.M. 1976. Glomerular permeability in disease: A proposed role of the glomerular epithelial cell. (*Abstr.*) *Proc. Am. Soc. Nephrol.* **9**:69
12. Carrie, B.J., Myers, B.D. 1980. Proteinuria and functional characteristics of the glomerular barrier in diabetic nephropathy. *Kidney Int.* **19**:669–676
13. Caulfield, J.P., Farquhar, M.G. 1976. Distribution of anionic sites in normal and nephrotic glomerular basement membranes. (*Abstr.*) *J. Cell Biol.* **70**:92*a*
14. Chang, R.L.S., Deen, W.M., Robertson, C.R., Brenner, B.M. 1975. Permselectivity of the glomerular capillary wall: III. Restricted transport of polyanions. *Kidney Int.* **8**:212–218
15. Chang, R.L.S., Deen, W.M., Robertson, C.R., Bennett, C.M., Glassock, R.J., Brenner, B.M. 1976. Permselectivity of the glomerular capillary wall. Studies of experimental glomerulonephritis in the rat using neutral dextran. *J. Clin. Invest* **57**:1272–1286
16. Chang, R.L.S., Robertson, C.R., Deen, W.M., Brenner,

B.M. 1975. Permselectivity of the glomerular capillary wall to macromolecules: I. Theoretical considerations. *Biophys. J.* **15**:861–886
17. Chang, R.L.S., Ueki, I.F., Troy, J.L., Deen, W.M., Robertson, C.R., Brenner, B.M. 1975. Permselectivity of the glomerular capillary wall to macromolecules: II. Experimental studies in rats using neutral dextran. *Biophys. J.* **15**:887–906
18. De Bats, A., Gordon, A.H., Rhodes, E.L. 1974. Variations in glomerular sialic acid content in diabetes and as the result of aging. *Clin. Sci. Molec. Med.* **47**:93–95
19. Deen, W.M., Bohrer, M.P., Brenner, B.M. 1979. Macromolecule transport across glomerular capillaries: Application of pore theory. *Kidney Int.* **16**:353–365
20. Deen, W.M., Bridges, C.R. 1982. Addenda and correction. Molecular charge of horseradish peroxidase. *Am. J. Physiol.* **242**:*F*750
21. Deen, W.M., Robertson, C.R., Brenner, B.M. 1972. A model of glomerular ultrafiltration in the rat. *Am. J. Physiol.* **223**:1178–1183
22. Deen, W.M., Satvat, B., Jamieson, J.M. 1980. Theoretical model for glomerular filtration of charged solutes. *Am. J. Physiol.* **238**:*F*126–*F*139
23. Deen, W.M., Satvat, B. 1981. Determinants of the glomerular filtration of proteins. *Am. J. Physiol.* **241**:*F*162–*F*170
24. Dubois, R., Decoodt, P., Gassèe, J.P., Verniory, A., Lambert, P.P. 1975. Determination of glomerular intracapillary and transcapillary pressure gradients from sieving data: I. A mathematical model. *Pfluegers Arch.* **356**:299–316
25. Eisenbach, G.M., Van Liew, J.B., Boylan, J.W. 1975. Effect of angiotensin on the filtration of protein in the rat kidney: A micropuncture study. *Kidney Int.* **8**:80–87
26. Galaske, R.G., Baldamus, C.A., Stolte, H. 1978. Plasma protein handling in the rat kidney: Micropuncture experiments in the acute heterologous phase of anti-GBM nephritis. *Pfluegers Arch.* **375**:269–277
27. Gassèe, J.P. 1973. Effect of acetylcholine on glomerular sieving of macromolecules. *Pfluegers Arch.* **342**:239–254
28. Gassèe, J.P., Dubois, R., Staroukine, M., Lambert, P.P. 1976. Determination of glomerular intracapillary and transcapillary pressure gradients from sieving data: III. The effects of angiotensin II. *Pfluegers Arch.* **367**:15–24
29. Hardwicke, J., Cameron, J.S., Harrison, J.F., Hulme, B., Soothill, J.F. 1970. *In:* Proteins in Normal and Pathological Urine. Y. Manuel, J.P. Revillard, and H. Betuel, editors. pp. 111–152. University Press, Baltimore
30. Huss, R.E., Marsh, D.J., Kalaba, R.E. 1975. Two models of glomerular filtration rate and renal blood flow in the rat. *Ann. Biomed. Eng.* **3**:72–99
31. Lambert, P.P., Aeikens, B., Bohle, A., Hanus, F., Pegoff, S., Van Damme, M. 1982. A network model of glomerular function. *Microvasc. Res.* **23**:99–128
32. Lambert, P.P., Dubois, R., Decoodt, P., Gassèe, J.P., Verniory, A. 1975. Determination of glomerular intracapillary and transcapillary pressure gradients from sieving data: II. A physiological study in the normal dog. *Pfluegers Arch.* **359**:1–22
33. Leber, P.D., Marsh, D.J. 1970. Micropuncture study of concentration and fate of albumin in rat nephron. *Am. J. Physiol.* **219**:353–363
34. Lui, S., Kalant, N. 1974. Carbohydrate of the glomerular basement membrane in normal and nephrotic rats. *Exp. Molec. Pathol.* **21**:52–62
35. Michael, A.F., Blau, E., Vernier, R.L. 1970. Glomerular polyanion: Alteration in aminonucleoside nephrosis. *Lab. Invest.* **23**:649–657
36. Oken, D.E., Cotes, S.C., Mende, C.W. 1972. Micropuncture study of tubular transport of albumin in rats with aminonucleoside nephrosis. *Kidney Int.* **1**:3–11
37. Olsen, J.L., Rennke, H.G., Venkatachalam, M.A. 1981. Alterations in the charge and size selectivity barrier of the glomerular filter in aminonucleoside nephrosis in rats. *Lab. Invest.* **44**:271–279
38. Pappenheimer, J.R. 1953. Passage of molecules through capillary walls. *Physiol. Rev.* **33**:387–423
39. Pappenheimer, J.R., Renkin, E.M., Borrero, L.M. 1951. Filtration, diffusion and molecular sieving through peripheral capillary membranes. A contribution to the pore theory of capillary permeability. *Am. J. Physiol.* **167**:13–46
40. Renkin, E.M., Gilmore, J.P. 1973. *In:* Handbook of Physiology, Section 8: Renal Physiology. J. Orloff and R.W. Berliner, editors. pp. 185–248. American Physiological Society, Washington
41. Rennke, H.G., Cotran, R.S., Venkatachalam, M.A. 1975. Role of molecular charge in glomerular permeability: Tracer studies with cationized ferritins. *J. Cell Biol.* **67**:638–646
42. Rennke, H.G., Patel, Y., Venkatachalam, M.A. 1978. Glomerular filtration of proteins: Clearance of anionic, neutral, and cationic horseradish peroxidase in the rat. *Kidney Int.* **13**:278–288
43. Rennke, H.G., Venkatachalam, M.A. 1977. Glomerular permeability: *In vivo* tracer studies with polyanionic and polycationic ferritins. *Kidney Int.* **11**:44–53
44. Rennke, H.G., Venkatachalam, M.A. 1979. Glomerular permeability of macromolecules. Effect of molecular configuration on the fractional clearance of uncharged dextran and neutral horseradish peroxidase in the rat. *J. Clin. Invest.* **63**:713–717
45. Robson, A.M., Giangiacomo, J., Keinstra, R.A., Naqvi, S.T., Ingelfinger, J.R. 1974. Normal glomerular permeability and its modification by minimal change nephrotic syndrome. *J. Clin. Invest.* **54**:1190–1199
46. Smith, F.G., III, Deen, W.M. 1980. Electrostatic double-layer interactions for spherical colloids in cylindrical pores. *J. Colloid Interface Sci.* **78**:444–465
47. Verniory, A., Dubois, R., Decoodt, P., Gassèe, J.P., Lambert, P.P. 1973. Measurement of the permeability of biological membranes: Application to the glomerular wall. *J. Gen. Physiol.* **62**:489–507
48. Winetz, J.A., Robertson, C.R., Golbetz, H.V., Carrie, B.J., Salyer, W.R., Myers, B.D. 1981. The nature of the glomerular injury in minimal change and focal sclerosing glomerulopathies. *Am. J. Kidney Dis.* **1**:91–98

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