Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability

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Invited Review

Polyp disperser mixtures of dextran, and more recently Ficoll, are frequently used as molecular probes in studies of glomerular permselectivity. After being filtered through the glomerular capillary wall (GCW), polysaccharides, unlike proteins, are left unreabsorbed by the proximal tubules. This implies that their sieving coefficients (θ), i.e., their filtrate-to-plasma concentration ratios, can be determined directly from their urinary clearances relative to that of a glomerular filtration rate marker (e.g., inulin). Infusing polyp disperser mixtures of dextran or Ficoll and using chromatographic techniques to fractionate plasma and urine samples make it possible to simultaneously determine the θ for a wide spectrum of different-sized molecular probes, provided that proper size calibrations are made. In humans, dextran can be used, whereas Ficoll, due to a toxic contaminant used for polymerization, cannot be used (in large quantities). Exceptionally, however, it has been given to humans in tracer quantities (1, 4). The technique of using polyp disperser polysaccharides as probes for glomerular permselectivity is remarkably reproducible, reliable, and elegant.

The sieving characteristics of the GCW can be modeled using simple pore models (37, 45, 51–53, 56), even though, recently, a number of seemingly more sophisticated models have been presented (16, 26, 44). Using the so-called “two-pore model” of glomerular permeability, the selectivity of the GCW is characterized by a very large number of albumin-restrictive “equivalent” small pores [radius (rₐ) = 37–55 Å], being negatively charged, and a small number of larger pores [radius ~100 Å (also negatively charged)], which constitute approximately only 1 part/million of the small pores (37, 56, 63). Using “neutral” dextrans as probes for testing the molecular selectivity of the GCW, data are usually compatible, with the presence of an equivalent rₑ being 48–60 Å (27). Using neutral Ficoll, a polymer of sucrose and epichlorohydrin, the rₑ has generally been determined to be on the order of 45–52 Å (4, 26, 30, 44, 46). However, massive data from classic micropuncture studies (53) and recent studies assessing the glomerular clearance of endogenous globular protein tracers in vivo (37) strongly indicate that the equivalent pore radius of the glomerular filter vis-à-vis neutral proteins would be only

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on the order of 37 to 38 Å (cf. Fig. 4 in Ref. 53; Fig. 1). This implies that size selectivity, rather than charge selectivity, would dominate the permselective properties of the GCW. Thus, typically, the dextran glomerular $\theta$ for a molecule with a Stokes-Einstein (SE) radius of 30 Å is approximately sevenfold higher than that of neutral horseradish peroxidase (nHRP) (55), a neutral globular protein of equivalent molecular radius. The glomerular $\theta$ value for a 30-Å Ficoll (Ficoll36Å) probe is characteristically 4 to 5 times larger than that of nHRP (1, 4, 30).

Current mathematical models describing restricted solute diffusion and filtration through a porous (or a fibrous) barrier assume the probe molecules to behave as rigid spheres. Dextran is a flexible, linear polymer of $\alpha$-glucopyranose, which, however, behaves like a random coil; i.e., it is far from a solid sphere. The suitability of dextran as a probe for testing glomerular permeability has therefore been seriously questioned (6, 16, 23, 37, 46, 47, 52, 53, 55). On the other hand, Ficoll, a markedly branched and cross-linked polymer of sucrose and epichlorohydrine has, due to its more spherical shape, been postulated to be a far better probe than dextran for determining the equivalent pore radius of the GCW (46). In fact, Ficoll molecules in solution are characterized by the lowest shape asymmetry of known polymers among nonglobular molecules (33). Like dextran, Ficoll is not reabsorbed by the proximal renal tubules, and hence its $\theta$, when assessed directly from urinary excretion data, can be set equal to its fractional glomerular clearance (i.e., $\theta$). The more ideal properties of Ficoll have thus been postulated to make its clearance more similar to that of globular proteins than is the case for dextran. The first indication of a lower glomerular permeability for Ficoll vs. dextran was provided by Bohrer et al. (6). Two subsequent studies confirmed this finding and yielded Ficoll $\theta$ that were actually, in some instances, lower than those obtained for globular proteins (46, 51) (Fig. 2). However, already the data of Bohrer et al. (6) indicated a higher permeability of the GCW for Ficoll than for neutral globular proteins, and in principle, all later studies have yielded markedly higher $\theta$ values and markedly higher equivalent $r_e$ of the GCW when determined with Ficoll than by using globular proteins (Fig. 2). In the present review, we will therefore challenge the concept that Ficoll would be an ideal molecule for probing glomerular permselectivity to (globular) proteins. This short overview will, however, not address the ambiguities inherent in, for example, size-exclusion chromatographic (SEC) techniques, column calibration, and detection of polydisperse polysaccharide preparations, which were discussed at some length in a previous publication (27).

**Fig. 1.** Glomerular sieving coefficients ($\theta$) simulated for proteins (37, 38, 53) (solid line), Ficoll (6, 26) (dotted line), and dextran (27) (hatched line) using pore theory of glomerular permselectivity (37, 63). Note the relatively large differences in “equivalent small-pore radius” obtained using the different sized molecular probes. For proteins, the equivalent small-pore radius is 37.5 Å, for Ficoll it is 46 Å, and for dextran it is 55 Å. The glomerular filtration rate is set to 3 ml/min, and unrestricted pore area over unit diffusion path length ($A_e/\Delta X$) is set to 1.8·10$^6$ cm.

**Fig. 2.** Shown are glomerular $\theta$ vs. the Stokes-Einstein (SE) radius for proteins (solid line) as summarized by Renkin and Gilmore (53) and Maack et al. (38) and assessed by Lund et al. (37) compared with selected data for Ficoll. The dotted-dashed line refers to recent measurements by Hjalmarsson et al. (28), which are identical to those by Bohrer et al. (6) and agree with the majority of Ficoll sieving measurements in the literature (1, 4, 25, 47), whereas the dotted line refers to measurements by Ohlson et al. (45), measured at a much lower $A_e/\Delta X$ than in the study by Hjalmarsson et al. (28). The data of Oliver et al. (46) deviate markedly from the bulk of measurements for Ficoll in the literature.

**COMPARISON OF GLOMERULAR $\theta$ OF UNCHARGED GLOBULAR PROTEINS AND GLOMERULAR $\theta$ OF POLYSACCHARIDES IN EXPERIMENTAL MODELS**

In a classic study by Rennke and Venkatachalam (55) in the rat, glomerular $\theta$ of nHRP was found to be 0.068 compared with a $\theta$ value of 0.483 for neutral dextran, yielding a dextran-to-HRP $\theta$ ratio of 7.3. In vivo (in humans) or in the isolated (cooled) artificially perfused kidney (cIPK), the $\theta$ for Ficoll36Å has typically been determined to be on the order of 0.08 to 0.10 and that for Ficoll40Å to be 0.01–0.04 (1, 4, 26, 28, 30). This is much higher than the corresponding values for proteins of equivalent hydrodynamic radius. Lund et al. (37) determined $\theta$ for neutral albumin in intact Wistar rats to be 0.006, which is 10-fold lower than the corresponding value for Ficoll. In the perfusion-fixed, isolated perfused kidney (iIPK), $\theta$ for neutral albumin was determined to be 0.020 (i.e., only 20% of that for Ficoll36Å) (13). Lindström et al. (36) determined the glomerular $\theta$ for lactate dehydrogenase-5 (LDH-5; radius of 40–42 Å and near-neutral charge) in cIPK (8°C) to be 0.006, which is again much lower than the corresponding value for Ficoll40Å.

Unfortunately, very few studies have been performed in which the Ficoll $\theta$ has been determined simultaneously with that for neutral globular proteins to elucidate the role of shape and
deformability, aside from size (and charge), in glomerular transport. Future studies of that kind are worthwhile.

IN VITRO POLYSACCHARIDE PERMEABILITY ACROSS POROUS MEMBRANES

For artificial membranes with track-etched pores, the diffusion of dextran and Ficoll has been analyzed according to conventional hydrodynamic pore models for diffusion of hard spheres in cylindrical tubes (7). In experimental systems with such porous membranes, the agreement between data and theory was poor for dextran, whereas it was much better for Ficoll. Dextran showed considerable hyperpermeability, and even when the ratio of molecular radius \( a_e \) to pore radius \( r_p \) \( a_e/r_p \) approached unity, dextran was able to pass through the membrane (7). In other words, dextran molecules were able to pass through membrane pores with a smaller diameter than the molecule itself. At all \( a_e/r_p \) values, dextran was found to diffuse more readily through the membrane than did Ficoll. However, at \( a_e/r_p \) values of 0.32 and 0.66, but not at lower values (15), Ficoll, as analyzed according to pore theory, also showed signs of hyperpermeability (2- to 3-fold) compared with the predicted behavior of an ideally hard sphere. Thus even though the behavior of Ficoll is apparently closer to that of a hard sphere than is that of dextran, both polysaccharides actually seemed to significantly deviate from the predictions of pore theory for a hard sphere in macroscopic (plastic) membranes in vitro, at least when the molecular radius approached the pore radius. Contrary to these results obtained for track-etched membranes, in a series of experiments using agarose gels, the diffusivities (31), \( \theta \) (32), and equilibrium-partitioning coefficients (34) of Ficoll were found to be almost indistinguishable from those of globular proteins of equivalent SE radius. In addition, charge effects were absent in those experiments. Thus polysaccharides and proteins seem to behave in a very similar fashion when interacting with gels in vitro, whereas this is not the case when the interaction occurs with track-etched porous membranes.

RELATIONSHIPS BETWEEN MOLECULAR WEIGHT AND EFFECTIVE (IN VITRO) MOLECULAR RADIUS FOR GLOBULAR PROTEINS, FICOLL, AND DEXTRAN

The minimal volume that a molecule can have is that of a hard, uniform sphere. For such a sphere, the radius \( a_e \) would theoretically be \( a_e = 0.67 \) [molecular weight (MW)]\( ^{0.333} \). This relationship is derived from the equation for the volume of a sphere, taking into account the molecular density for an unhydrated protein molecule. In principle, the molecular density is the inverse of the “specific molal volume” of the molecule, being 0.70–0.75 cm\(^3\)/g for proteins. Hence, the molecular density for a globular protein would average 1.33 g/cm\(^3\). The effective molecular radius can thus be solved from the equation

\[
MW = \frac{4\pi(a_e)^3}{3} \rho \cdot N_A \tag{1}
\]

where \( N_A \) is Avogadro’s number (6.02\( \cdot 10^{23} \)), and \( \rho \) is the density of the molecule, here being set at 1.33 g/cm\(^3\). Rearranging Eq. 1 and inserting the appropriate values for \( \pi, \rho, \) and \( N_A \) yield the expression (in \( \AA \)) for \( a_e \)

\[
a_e = \frac{3MW^{0.333}}{4\pi\rho N_A} = 0.67\ (MW)^{0.333} \quad \tag{2}
\]

For a hydrated protein, the molecular density would be reduced to 0.99 g/cm\(^3\), assuming a hydration factor for protein of 0.3 g/g protein (10). For a polysaccharide, such as Ficoll, the molecular density is \( \sim 1.0–1.1 \) (33). In Fig. 3, the relationship of an effective \( a_e \) on the ordinate is plotted vs. the MW on the abscissa in a log-log diagram for hard, uniform spheres (bold solid line); for hydrated hard spheres (bold hatched line; from Eq. 2, in which \( \rho \) is set at 0.99 g/cm\(^3\)); and, based on literature data, for a number of globular proteins (open circles and blue solid line) and Ficoll (green line) and dextran (red and brown lines). The following equations have been used to relate the SE radius \( a_e \) in \( \AA \) to the MW (in Da)

For a hard sphere:
\[ a_e = 0.67 \ (MW)^{0.333} \quad \text{(theoretical; black line)} \]

For a hydrated hard sphere:
\[ a_e = 0.74 \ (MW)^{0.333} \quad \text{(theoretical; black, hatched line)} \]

For globular proteins:
\[ a_e = 0.483 \ (MW)^{0.386} \quad \text{(best fit; blue line; Ref. 57)} \]

For Ficoll:
\[ a_e = 0.421 \ (MW)^{0.427} \quad \text{(green line; Ref. 46)} \]

For monodisperse dextran:
\[ a_e = 0.488 \ (MW)^{0.437} \quad \text{(red line; Ref. 46)} \]

For polydisperse dextran:
\[ a_e = 0.33 \ (MW)^{0.463} \quad \text{(brown line; Ref. 22)} \]

As seen in Fig. 3, the empirical SE radii of all “real” molecules fall above the theoretical relationship for a hard, uniform sphere, and even for a hydrated hard sphere. This is...
probably due to the fact that molecules are not ideal (hydrated) spheres; i.e., there is an extension of molecular structure. However, for any given MW, the effective molecular “density” conceivably appears to be lower for Ficoll, and much lower for dextran, than that generally for globular proteins of equivalent SE radius. Thus whereas dextran seems to deviate the most from uniform hard spheres, partly due to large shape asymmetry, there is also a considerable deviation for Ficoll. This cannot be explained by an asymmetric configuration of Ficoll, because Ficoll has low shape asymmetry. Instead, the plot in Fig. 3 indicates that Ficoll is markedly less dense than any compact globular protein. Another interpretation, in line with data presented below, may be that Ficoll and dextran are more deformable (flexible) than globular proteins in general. However, this reasoning ignores (1) differences in polymer chain stiffness due to different types of intramolecular bonds and (2) any cross-links (and their nature and type) that may be present. By contrast, molecular density differences due to different atomic compositions are, as mentioned above, relatively small among proteins and polysaccharides.

If polysaccharides have a more flexible structure, or are far more extended (cf. dextran), than are globular proteins, this may be a major reason for the discrepant pore radius estimations obtained with either kind of molecular species in glomerular sieving experiments. A discrepant pore radius estimation can also result from the use of other extremely asymmetric molecules of a polysaccharide nature, such as glucosaminoglycans (GAGs), or from the use of molecules having a large polysaccharide component, such as glycoproteins. In Fig. 3, we have included a highly asymmetric GAG, namely, low-MW hyaluronan (HA) of 12,000 MW (grey circle) and also bikunin (grey circle), a (negatively charged) glycoprotein having a MW of 8,000, and also the highly asymmetric protein myosin (grey circle above the dextran line). HA (MW 12,000) and bikunin (MW 8,000) have in vitro, due to their asymmetric shape, and due to their content of linear polysaccharides, an SE radius equivalent to that of albumin. Myosin also falls far above the relationship for dextran. In fact, these highly asymmetric molecules deviate even more markedly than do Ficoll and dextran from the theoretical curve for rigid, uniform (hydrated) spheres. Indeed, Ohlson et al. (45) showed that HA (MW 12,000) and bikunin, although being negatively charged, showed a markedly higher glomerular permeability in IPK of rats than did uncharged Ficoll_{36k} and native albumin. The θ of (negative) HA and bikunin (and of uncharged Ficoll_{36k}) were also much larger than that for the neutral globular molecule LDH5 (radius of 40–42 Å; θ = 0.006). This indicates that shape asymmetry actually can surpass the impact of molecular charge on glomerular transport. The generalization that can be made from Fig. 3 is that the more a molecule’s SE radius deviates from the theoretical line for a hydrated hard sphere (solid black line), the more “abnormal,” according to published data, is its glomerular permeability.

**DOES FICOLL BEHAVE LIKE A COMPACT MOLECULE?**

Recent physicochemical data suggest that Ficoll, which is an extremely branched polysaccharide, does not behave like a compact molecule. When dissolved in a NaCl solution of physiological ionic strength (0.15 M) or in Na phosphate buffer (at 0.1–0.25 M), its intrinsic viscosity, compared with the situation at low ionic strength (0.0015 M), has been found to increase by ~15%, thus indicating an expansion of the molecule (59, 66). It seems that Ficoll, partly via interactions between (weakly charged) groups within the interior of the molecule and Na⁺ ions, when dissolved in body fluids, transforms from a hard-packed sphere to a rather deformable molecule. Experiments have thus shown that the Ficoll molecule can adsorb Na⁺ ions (or K⁺ ions), whereby Ficoll takes on the properties of a pseudopolyocation (59, 66). Further evidence that Ficoll has a rather open, deformable structure comes from “crowding” experiments, in which the intrinsic viscosity and excluded volume have been measured as a function of solution concentration (67). At increasing Ficoll concentrations up to 2.5%, there was an increase in the so-called “excluded volume” in the solution, owing to intermolecular interactions of the Ficoll molecules, i.e., due to molecular crowding. However, at concentrations of >2.5% the relative excluded volume was again reduced. This drop in excluded volume is most likely the result of molecular “compression” of the highly branched Ficoll particles. Thus the behavior of Ficoll in crowded solutions strongly suggests that the Ficoll particles have a much more “open” structure than has been previously commonly conceived.

**CONSEQUENCES OF POLYSACCHARIDE DEFORMABILITY ON DETERMINATIONS OF GLOMERULAR PERMSELECTIVITY**

There are three kinds of deformation of polymers: 1) random fluctuations in size or shape due to thermal motion, 2) flow-induced perturbations of the average size or shape, and 3) changes in average size or shape due to variations in solution composition (such as ionic strength). With respect to glomerular permeability, random fluctuations in size or shape due to thermal motion are most relevant, because rates of shear or extension are orders of magnitude too small to be of importance (17). Evidence cited above (59, 66) tends to support deformability of the third kind. However, the crowding experiments mentioned earlier may support deformability according to the first.

Whereas the hyperpermeability of dextran in experimental settings is well documented, recent chemical and physical data thus indicate that Ficoll molecules are indeed not acting like hard spheres but rather like deformable molecules. The theoretical consequence of the deformability of polysaccharides like Ficoll or dextran is that these molecules, when used in studies of glomerular permselectivity to proteins, evidently yield a higher rₚ than do globular proteins. If Ficoll is compressible, as indicated from physical studies, it is conceivable that the pore radius would be “overestimated” in Ficoll-sieving experiments relative to protein-sieving experiments in vivo (37). Alternatively, it may be the protein-sieving data that “underestimate” the rₚ. (37). At low ionic strength, Ficoll would theoretically be more similar to proteins as a probe for glomerular permeability, because it would then be much more compact. This is in line with measurements in cIPK, where Sörensson et al. (61) experimentally demonstrated a lower rₚ (41 Å) for Ficoll when the ionic strength was reduced, compared with conditions of physiological ionic strength (rₚ = 45–46 Å).

In a number of pathophysiological conditions, in which the selectivity of the glomerular filter is reduced by the apparent
formation of “large pores” or “shunt pathways,” Ficoll36Å, due to its relative hyperpermeability, actually has often failed to increase its θ (1, 47). In the same situation, the θ of native (negative) albumin, the transport of which (according to the 2-pore theory) would be more or less totally confined to the large pores, is usually dramatically enhanced. Because of the marked increase in θ for albumin but the failure of Ficoll36Å to reflect small changes in glomerular size selectivity, the conclusion has often been that charge selectivity, instead of size selectivity, has been altered. However, taking into account the discrepant size-selective properties of Ficoll vs. proteins, these data may be reinterpreted, at least partly, as reflecting changes in size selectivity.

ARE NEUTRAL DEXTRAN AND FICOLL COMPLETELY UNCHARGED?

The general belief among chemists today is that neutral dextran and also Ficoll actually carry a very weak negative charge (66). Despite this, it is usually assumed that these polysaccharides, especially Ficoll, are completely electrically neutral. However, because Ficoll is highly branched and cross-linked, it has numerous end groups. Thus the probability that at least a few of them have undergone oxidation to form carboxylic acid residues is quite high. Such carboxylic groups can interact with Na+ ions, which endow the molecule with properties of a weak polycation (see DOES FICOLL BEHAVE LIKE A COMPACT MOLECULE?). Indeed, recent SEC results indicate that the Ficoll molecule behaves like a weakly charged particle (50, 59). It thus shows adsorption behavior to porous glass media (e.g., Corning porous glass) used as the stationary phase in SEC columns. It cannot be excluded, however, that the adsorption of Ficoll may have occurred due to van der Waals forces and is not charge related. It is intriguing that Ficoll has actually been used to test glomerular permselectivity under conditions when the ionic strength has been varied, assuming that the Ficoll molecule does not change either its surface charge or its molecular structure under those conditions (60). Overall, however, the apparent weak charge of the Ficoll molecule should per se not significantly affect its glomerular sieving behavior. It is the conformational changes (molecular expansion when ionic strength is increased), secondary to the intramolecular end groups, some of which are charged, that are of importance.

CHARGE SELECTIVITY OF THE GLOMERULAR FILTRATION BARRIER VIS-À-VIS PROTEINS OR POLYSACCHARIDES

During the 1970s, it became established that the glomerular filter discriminates among macromolecules based on both their size as well as their net charge (5, 9, 11, 39). Evidence in favor of charge selectivity of the glomerular filter was based on comparisons of sieving data for uncharged vs. anionic dextran, in the form of sulfated dextran, and uncharged vs. polycationic (DEAE) dextran. Dextran sulfate was thus found to be markedly retarded compared with neutral dextran. Furthermore, a facilitated filtration of DEAE dextran was demonstrated in normal Munich-Wistar rats (5). In rats affected by nephrotic serum nephritis, charge selectivity was much less pronounced than in control rats (5).

These studies have been criticized, however, on the grounds that sulfated dextran may bind to, and be even processed by, glomerular cells (62, 68), and further that dextran sulfate can bind to plasma proteins (23). In addition, isolated glomerular basement membranes have generally failed to show charge selectivity when probed with neutral and negatively charged Ficoll (8) or native (anionic) or cationized albumin (3). In line with these results, Schaeffer et al. (58) were also not able to find any difference in θ for differently charged polysaccharides, as assessed using fluorescently labeled neutral and negatively charged, nonsulfated dextrans, or neutral and negatively charged (nonsulfated) hydroxyethyl starch (HES). The HES molecules, furthermore, showed lower θ for any given in vitro molecular radius than did dextran (cf. Ficoll). It was concluded that the glomerular filtration barrier restricts the transport of polysaccharide macromolecules as a function of their size and configuration but not due to the presence or absence of a negative charge. A similar conclusion was recently reached by Guimarães et al. (25), comparing negatively charged, carboxymethyl Ficoll with native Ficoll in rats. There was no significant difference in the θ of the differently charged Ficoll species. For molecules with radii greater than 36 Å, negatively charged carboxymethyl Ficoll had even a facilitated clearance compared with uncharged Ficoll (25).

In stark contrast to the apparent inability of the glomerular filter to discriminate between polysaccharides of different charges, apparent from recent literature data as cited above, there is ample evidence that, indeed, the GCW selects globular proteins based on their charges. This has been extensively reviewed by Comper and Glasgow (14). Rennke et al. (54) showed a significant reduction (by a factor 1/8.7) of the clearance of negatively charged HRP [isoelectric point (pI) <4.0] compared with neutral HRP (pI 7.3–7.5). Furthermore, cationic HRP (pI 8.4–9.2) had a sevenfold higher clearance than neutral HRP. A tissue-uptake technique was used, and HRP was assessed by an enzymatic technique. Therefore, the studies of Rennke et al. have later been criticized due to the possibility that enzymatic activity may underestimate the level of HRP and that the anionic material may be degraded during filtration (48). Still, the authors claiming these confounding circumstances found a difference in θ for nHRP vs. anionic HRP of approximately threefold, even when compensating for such shortcomings. A similar difference has been seen for anionic neutral amylase (19) and, in the cIPK, for nHRP and anionic HRP (36). In the fIPK, the difference in measured θ for native (negatively charged) albumin (8.7·10−3) and that for cationic albumin (20·10−3) was also approximately threefold (13). In the fIPK, the baseline θ for native albumin was, however, much higher than values obtained either in vivo using a tissue-uptake technique (37), in the cIPK (44), or in intact rats using a careful micropuncture technique (64). This indicates the presence of a significant size-selectivity defect under baseline conditions in the fIPK. Hence, in intact kidneys with a higher size selectivity to native proteins than in the fIPK, Lund et al. (37) found a ratio of θ of ~7–10 between neutral and native (negatively charged) albumin, later confirmed by Bakoush et al. (2). Similarly, high values were obtained for the ratio of the largely neutral probe LDH-5 to the negatively charged LDH-1 in the cIPK, the ratio averaging 7.59 (range 4.11–11.94) (61). On the other hand, studies comparing the clearances of neutral IgG2 to anionic IgG4 (pI 5.5–6.0) have yielded results indicating a lower degree of charge selectivity, with IgG2/IgG4 θ ratios varying from 1.35 (21) and 4 (65) up to 10 (18). Because of the much larger pore radius (~90–100
higher molecular deformability. In conclusion, the general pattern emerging with respect to charge selectivity of the glomerular filter is that 1) anionic proteins are always retarded compared with their neutral or cationic counterparts; 2) charge effects are relatively small among differently charged polysaccharide molecules, such as Ficoll or HES; and 3) neutral proteins have significantly lower clearances than dextran or Ficoll of equivalent hydrodynamic radius (nHRP 0.062, Ficoll 0.2, dextran 0.4; neutral albumin 0.006, Ficoll 0.08, and dextran 0.19; neutral LDH 0.0056, Ficoll 0.02, and dextran 0.04). In general, the θ of neutral proteins is in most cases just 3- to 10-fold higher than that of their negatively charged counterparts. This indicates that, for globular proteins, size selectivity may be of greater importance, and charge selectivity of lesser importance, than previously conceived for their sieving properties across the GCW (14). There is thus reason to believe that charge selectivity has generally been somewhat overrated over the last few decades, simply due to the fact that comparisons between uncharged and charged species have in many instances been made among molecules with discrepant configuration and deformability. When globular proteins have been compared with polydisperse polysaccharides, one has generally assumed that these two different classes of chemical compounds are similar with respect to size, configuration, and flexibility, which is evidently not the case.

We can only speculate why the GCW exhibits such a low selectivity difference with respect to differently charged polysaccharides, when it evidently separates proteins of different molecular charges. Does the process of charge modification of Ficoll, for example, lead to a higher degree of deformability or shape asymmetry of the molecule? Indeed, in the cIPK shape asymmetry for polysaccharide or GAG species, such as hyaluronan and bikunin (SE radius of 36 Å and negatively charged), greatly influences their glomerular transport (45), which was markedly increased compared with neutral Ficoll of an equivalent SE radius. Thus shape asymmetry markedly exceeded the importance of negative charge in these experiments. The present review proposes that deformability is another major factor critically influencing the molecular permeability across the GCW. Although Ficoll36Å has a shape somewhat closer to a sphere than does albumin, it is approximately one order of magnitude more permeable across the GCW in vivo than is neutral albumin (37), conceivably due to a much higher molecular deformability.

**CALIBRATION OF MOLECULAR SIZE TO MW**

How is one to deal with the problem of standardizing in vivo glomerular permeation of polysaccharides to in vitro assessments of molecular size (SE radius)? One way would be to replace the SE radius with so-called “hydrodynamic volume,” i.e., the MW times the intrinsic viscosity of the molecule ([η]·MW) (20, 40). In Fig. 4, the θ data of Bohrer et al. (6) for dextran and Ficoll in rats have been plotted vs. [η]·MW. Even though there seems to be a slightly better correspondence between polysaccharides and proteins with respect to glomerular permselectivity, Ficoll and dextran molecules still seem to be “hyperpermeable” compared with proteins, especially for molecules >30 Å in radius. We are thus again facing the problem that the sieving curves for dextran and Ficoll on the one hand and proteins on the other are different, conceivably due to differences in molecular deformability and shape. Dextran molecules deviate with respect to both shape and deformability, but those of Ficoll deviate conceivably due to an increased deformability compared with proteins.

**LIMITATIONS OF USING PROTEINS AS MOLECULAR PROBES FOR GLomerULAR PERMSELECTIVITY**

Experimental studies of glomerular protein transport cannot be performed in humans unless proximal tubular protein reabsorption is inhibited (41) or investigations of patients with Fanconi’s syndrome (e.g., Dent’s disease) are done (42). In animal models, such as the cIPK, proximal tubular reabsorption has been inhibited by tissue cooling, perfusion fixation (13, 26, 28, 30, 44, 45, 50, 61), or the use of inhibitors of tubular reabsorption, such as lysine, maleate, or cytochalasin B (12, 41). A large number of assessments of θ for proteins in micropuncture studies exist (53), but they have been criticized because they imply exposure and mechanical interactions with an intact kidney. Furthermore, protein samples from the tubules may bind to the glass pipette, and interstitial proteins may leak into the tubules during micropuncture. Moreover, the tubular micropuncture procedure has to be done at sites distally to Bowman’s capsule, and therefore primary urine cannot be directly assessed. Tubular protein concentration thus falls along the distance of the proximal tubule, because protein reabsorption is usually more avid than that of water. In an attempt to avoid all these sources of errors, Tojo and Endou (64), using a double-barrel pipette technique, avoided contamination from interstitial proteins. Furthermore, they assessed the tubular concentration of proteins together with that of a filtration marker (inulin) at various distances from Bowman’s capsule. With this technique, they were able to quite precisely estimate the urinary albumin protein concentration in Bowman’s capsule and in the proximal tubule. With this careful technique, they estimated the θ value from native albumin to be...
6.2 \times 10^{-4}$, which is in agreement with recent data obtained by a tissue-uptake technique in intact rat kidney or data using the cIPK (26, 37). Recently, the possibility of degradation of proteins by peptidases in renal proximal tubular cell brush borders has been indicated, at least after injection of (exogenous) radiolabeled albumin (24). Using a tissue-uptake technique and employing short periods of tracer sampling (short-term tracer accumulation) while recovering all radioactivity in urine and tissue (37) and/or preventing protein degradation by enzyme inhibition via cooling (30, 44, 45, 60), however, still yield a very low $\theta$ for albumin. Furthermore, proteomic analysis of normal and Fanconi urine yields no evidence for significant excretion of plasma protein fragments in normal urine (43).

In an assessment of $\theta$ for proteins using either micropuncture techniques, tissue-uptake techniques, or sieving experiments at low temperature (cIPK), only a few proteins of defined radii can be assessed. Furthermore, in such studies care should be taken to avoid protein-protein interactions, dimer formation, or binding to other proteins (cf. albumin) or to lipids. Also, the fact that even a “neutral protein” actually carries an equal number of positive and negative charges would surround such molecules with a mosaic of positively and negatively charged diffuse double layers. How such a complex double-layered structure would have an impact on the entry of such a (heterogeneously charged) molecule into a pore is not readily predicted.

The beauty of using polydisperse, inert polysaccharides as probes for glomerular permselectivity, besides avoiding the problems of using protein probes, is that a continuous range of molecular radii (from 15 up to 90 Å) can be assessed simultaneously, provided that proper size calibrations are made. The wealth of information from each experiment is great. Dextrans can be used in humans, whereas Ficoll, provided that minute tracer quantities are given, may also be used in clinical experiments (1, 4). It would thus be feasible to use dextran or Ficoll to probe glomerular pore characteristics in patients with pathophysiological glomerular alterations. However, because proteinuria is the clinical syndrome, and proteins show different sieving characteristics compared with polysaccharides, it would be desirable to find a way of converting glomerular sieving data for dextran and Ficoll into protein sieving characteristics. Thus it is the sieving property of the GCW vis-à-vis proteins that is the most relevant parameter clinically. The possibility that it is glomerular protein sieving that is actually abnormal and that the glomerular sieving of Ficoll, for example, is the one yielding a correct estimation of the real glomerular size selectivity cannot, however, be completely ruled out.

CONCLUSIONS

In summary, there is now good evidence that polysaccharide molecules, especially dextrans, are more permeable across the GCW than are neutral proteins at similar in vitro SE radii. Furthermore, negatively charged proteins are retarded compared with neutral ones. Since the 1970s, it has been suggested that size and charge are the major determinants of solute permeability across the GCW. However, it has become increasingly evident over the last two decades that shape and deformability are also of crucial importance. Polysaccharides, such as dextrans, have a looser structure, combined with a higher degree of deformability, which render them more permeable across the GCW in vivo than globular neutral proteins of equivalent in vitro SE radii. Recent data from crowding experiments and measurements of intrinsic viscosity further indicate that Ficoll may also exhibit “nonideal” properties due to a rather open, deformable structure and thus that it deviates from an ideally hard sphere. The discrepant sieving behavior across the GCW of dextran and Ficoll vs. proteins may indicate that the glomerular filter is actually acting more like a rather nondeformable membrane than a flexible gel.

To evaluate proteinuric conditions in, for example, experimental animal studies, it would thus be of value for an assessment of glomerular permselectivity to also use globular proteins as molecular probes, besides polysaccharides such as Ficoll or dextran. Polysaccharides are more deformable and, in the case of dextran, more asymmetric, than most globular proteins. Studies of glomerular protein transport are, however, problematic, because they require careful micropuncture or tissue-uptake techniques in animal models. For testing glomerular permeability in humans, we are still left with the only option available, namely, the assessment of dextran (or Ficoll) $\theta$. When probing glomerular permselectivity in humans, however, one should be aware of the relatively marked hyperpermeability that polysaccharides exhibit compared with globular proteins with equivalent in vitro SE radii. It would thus be worthwhile to try to translate the GCW sieving characteristics obtained for dextran and Ficoll into protein $\theta$. After all, it is the abnormal handling of proteins by the GCW, rather than that of polysaccharides, that is the physiologically relevant abnormality in proteinuric conditions.

ACKNOWLEDGMENTS

We are grateful to Kerstin Wihlborg for skillful typing of the manuscript.

GRANTS

This study was supported by Swedish Medical Research Council Grant 08285, the Lundberg Medical Foundation, and European Union Contract FMRX-C98-2019.

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