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EDITORIAL REVIEW

Charge selectivity in kidney ultrafiltration

Efforts at understanding the apparent charge selective nature of transglomerular transport of charged macromolecules, particularly through the work performed in the 1970's and 1980's have formed the basis for discussion of the properties of the glomerulus and its molecular components in health and disease. Central to this discussion is the role of the anionic sites provided by glycosaminoglycans and sialoglycoproteins in the extracellular matrices and on cell surfaces of the glomerular capillary wall (GCW). Recent studies suggest that some of the interpretations are in doubt and that the original studies should be revisited.

Anion sites of the glomerular capillary wall

Based on histochemical studies with cationic tracers, and enzymatic and chemical extraction techniques, it is widely accepted that the glomerular basement membranes (GBM) contain an array of anionic sites (>80%) due to the presence of heparan sulfate proteoglycan [1–4]. Smaller amounts of chondroitin sulfate proteoglycan and hyaluronan have been detected [5]. Similarly, the major anionic sites in the mesangial matrix are provided by heparan sulfate and a lesser proportion by chondroitin sulfate proteoglycans [6, 7]. Epithelial and endothelial cell surfaces are known to retain heparan sulfate [8, 9]. The foot processes, too, are coated with sialoglycoproteins that will contribute anion charge [10].

Filtration characteristics

The permeability of the glomerular capillary wall to macromolecules is quantitatively expressed in terms of fractional clearance. It represents the relative transport of the macromolecule in question to the transport of water (equivalent to the glomerular filtration rate). Water transport is measured through the use of a molecule that is biologically inert and is freely filtered by the glomerulus such as inulin or creatinine. Experimental measurements of fractional clearance have employed labeled macromolecules (or more recently used specific antibodies) (Table 1) in either single nephrons *in situ* or in whole kidneys (both *in vivo* or *ex situ*) where the labeled material is estimated in the urine. Fractional clearance estimations from urine samples have to take into consideration the possible post-glomerular tubular uptake of the macromolecule.

Size selectivity

Fractional clearance studies to measure the size selectivity associated with glomerular filtration have universally employed dextran as a test transport probe. It is neither secreted nor reabsorbed by the renal tubules so its clearance is easily measured. The early studies of size selectivity have been reviewed by Renkin and Gilmore [11]. The fractional clearance of dextran of different sizes by Chang et al [12] is shown in Figure 1. In these experiments a tritium-labeled dextran fraction with a wide molecular weight distribution was intravenously administered to rats to a steady state level (plasma concentration ≤10 mg/100 ml) where blood plasma and urine fractions were collected and analyzed for radioactivity on Sephadex G100 (in 0.05 N NH_4Ac) precalibrated for hydrodynamic radii. For a given hydrodynamic size, the relative amounts of labeled dextran in the urine and in plasma will determine the fractional clearance value. A value of unity for the fractional clearance corresponds to an equal clearance of dextran and inulin. That is, any molecule with molecular radii ≤18 Å will be filtered freely across the glomerular wall. A value of zero corresponds to complete impermeability of the molecule. Chang et al [12] concluded from the data shown in Figure 1 that the normal glomerular capillary wall acts as a membrane with uniform pores with radii of approximately 50 Å; the pores themselves have yet to be identified. While the fractional clearance approaches zero for radii ~42 Å more recent studies have demonstrated finite clearances for dextrans up to a radius of 60 Å. The size selectivity, as measured by the dextrans, is similar for dogs, humans and rats although marked diffences between different studies seem to occur at fractional clearances <0.01. Other test random coil molecules like polyvinylpyrrolidone show similar behavior to dextran. The caution that should accompany these studies is that molecular sizes are estimated in solvent environments in column chromatography that are very different to those conditions these molecules would experience in plasma.

Fractional clearance of proteins

A major function of the glomerular capillary wall is to severely restrict the transglomerular passage of albumin and other plasma proteins while filtration is occurring. In normal kidneys, some albumin transport across the capillary wall may occur and it is then subject to endocytosis by tubular cells. Therefore, the appearance of protein in urine is the result of two processes: a dominant filtration rejection at the capillary wall and postglomerular scavenging by tubules.

Studies on the fractional clearance of albumin, whose radius is normally quoted at 36 Å (see, however, next section), have yielded a range of values, all of which are considerably lower (by a factor <1/20) than dextran of equivalent hydrodynamic size (fractional clearance ~0.1; Table 2). The fractional clearance of albumin would make it equivalent to a dextran molecule with a radius ~43 to 60 Å. The wide range of equivalent radii again reflects the differences between various laboratories in terms of their fractional clearance data. For example, if it is assumed that the fractional clearance of albumin is 0.003 then this would correspond to dextran of radii of 43 Å (Fig. 1) or a range of 51 to 61 Å as studied in rats [18, 19], 54 Å as studied in rabbits [20], or a range of 60 to 64 Å as studied in humans [21–23]. In general, the comparative studies really typify the most extraordinary and

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Table 1. Commonly used molecular transport probes

Probe	Shape	Valence
Dextran	Random coil (with small stubbs of saccharide branching)	0
Ficoll	Spherical	0
Dextran sulfate	Random coil	~−60 (for radius of 36 Å)
Native horseradish peroxidase	Globular	0
Anionic horseradish peroxidase		−11

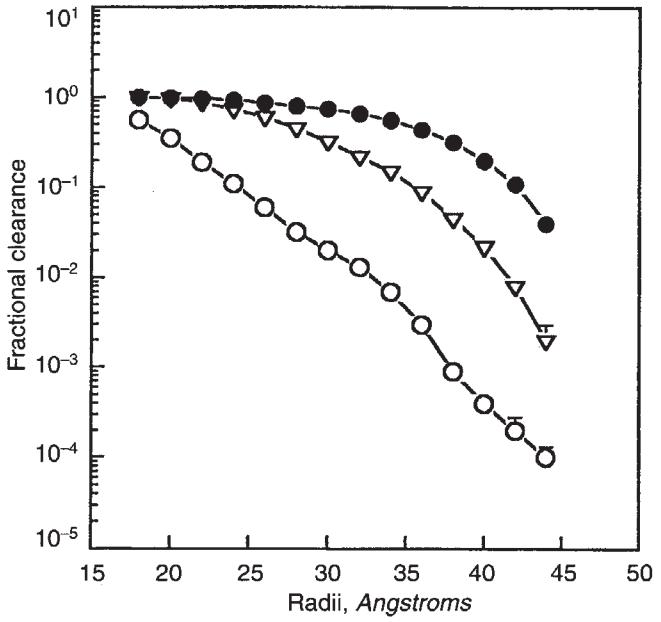

Fig. 1. *Fractional clearance of differently charged dextrans.* Fractional clearances in rats of DEAE dextran (●), dextran (▽), and dextran sulfate (○) as a function of effective hydrodynamic radii as determined by gel chromatographic analysis. Data have been obtained from [42].

unexplained aspect of the glomerular processing of albumin and other proteins in that they appear larger to the glomerular filter as compared to random coil molecules such as dextran. Because the filtration of albumin is restricted to a greater degree than would be predicted on hydrodynamic size alone, other factors have been investigated.

Serum albumin structure

Table 3 lists some pertinent physical characteristics for bovine, human and rat albumins. The albumin molecule is markedly polar with a potential for 100 negative and 82 positive charges. The overall net charge at pH 7 is −12 to −18 for the three albumins. The conformation of the molecule is not a sphere as is commonly stated in the literature but one of a prolate ellipsoid with the minor and major axis approximately 40 Å and 140 Å, respectively, and an axial ratio of 3.5. The molecule is essentially like a stubby cigar.

Table 2. Fractional clearance of albumin in rats

Fractional clearance	Reference	Technique to account for tubular reabsorption
0.0006	[13]	Tissue accumulation method[a]
0.005–0.007	[14]	No tubular reabsorption
0.001	[15]	Isolated perfused kidney, 10–20 min perfusion, Bowmans space/plasma ratio
0.003	[16]	Tissue accumulation method[a]
0.0003	[17]	Bowmans space/plasma ratio

[a] This method assumes that the marker present in the saline-flushed filtering kidney after an intravenous infusion represents the reabsorbed fraction of filtered marker as compared to a nonfiltering kidney

Table 3. Physicochemical properties of albumins (from Peters [24])

Property	Bovine	Human	Rat
Molecular weight	66267	66439	65871
Net charge pH 7	−18	−15	−12
Overall dimensions Å	41.6 × 140.9	38 × 150	

Role of conformation of the transport probe

It is often stated in the literature that a contributing factor to the glomerular permeability difference between dextran and albumin is the fact that dextran, because of its deformable compliant structure, may reptate (that is, extend and snake through the pores of the filter) through the GBM and therefore appear smaller on a hydrodynamic basis than albumin. There is, however, no direct evidence for this suggestion of the role of conformation.

Perhaps the most commonly cited evidence for the reptation effect in the renal literature comes from the data of Laurent et al [25] on the diffusion (not forced flow as described on occasion) of linear polymers with molecular weights between 30,000 and 530,000 in hyaluronan solutions at concentrations where there are continuous intermolecular interactions (transient networks). The diffusion study demonstrated that the linear molecules were less retarded in the hyaluronan network than globular particles of equal hydrodynamic dimensions. This interpretation has subsequently been put into doubt as the diffusion systems studied, particularly hyaluronan networks, are subject to gravitational instabilities [26]. These instabilities lead to ordered microconvection that may manifest apparent diffusion rates much faster than expected [27].

The onset of relative faster transport for linear molecules across porous membranes seems to occur only above a critical concentration of the diffusing molecule, which is probably related to concentration regimes where effective intermolecular interaction occurs, and is dependent on the partitioning at the solution/pore interface [28]. Whether these conditions are met in the fractional clearance studies of dextrans is not known but they are probably not.

Some studies have directly investigated the role of conformation on transglomerular transport. Rennke and Venkatachalam [29] found a difference in the fractional clearance of the globular horseradish peroxidase (HRP) (radius = 29.8 Å; 7-fold lower) as compared to equivalent sized dextran. Bohrer et al [30] compared the fractional clearance of polydisperse dextran with that of a more rigid, spherical molecule Ficoll (a cross linked copolymer of sucrose and epichlorohydrin). In the size range of 22 to 47 Å the

Table 4. Influence of charge on the fractional clearance of proteins

Probe	pI	Molecular weight	Radius Å	Fractional clearance	Tubular reabsorption correction	Reference
Native HRP	7.3–7.5	40,000	29.8	0.061	a	[32, 33] (rats)
Anionic HRP	<4.0	40,000	31.8	0.007	a	
Cationic HRP	8.4–9.2	40,000	30.0	0.338	a	
Pancreatic amylase	7.0	56,000		0.03	c	[36] (human)
Salivary amylase	5.9–6.4	56,000		0.01	c	
Native albumin	4.9	69,000	35	~0.006	c	[14] (rats)
Neutral albumin	5.5–6.6	69,000	33	~0.006	c	
Cationic albumin	7.2–8.2	69,000	33	~0.008	c	
Native albumin	4.9	69,000		0.0006	b	[13] (rats)
Cationic albumin	7.5–8.0			0.026	b	
Anionic IgG	4.9	150,000	52	0.004	a	[16] (rats)
Neutral IgG	7.4–7.6	150,000	52	0.001–0.005	a	
Ceruloplasmin	4.9	137,000	50	0.021	a	
Albumin	4.9	69,000	36	0.003	a	

Abbreviation is HRP, horseradish peroxidase.
[a] Tissue accumulation method without correction for binding (see Table 1)
[b] Tissue accummulation method with correction for binding (see Table 1)
[c] Negligible tubular reabsorption

dextran appeared more readily filtered than the Ficoll with a maximum difference (factor of 2) at radius of 36 Å. In a more recent and important study, Oliver et al [31] confirmed that Ficoll had a significantly lower clearance than dextran; for a radius of 36 Å dextran clearance was ~0.079 whereas the clearance for Ficoll was ~0.007. The plasma Ficoll concentration was not published in either study and its effective size under the experimental conditions was not measured. The authors found it difficult to explain, in physical terms, the differences in clearance as the transport of random coil molecules as compared to Ficoll through inert synthetic membranes does not consistently manifest the same type of behavior. It was argued, however, that the results for Ficoll go a long way in accounting for the difference between the clearance of dextran and albumin.

The conclusion from all these experiments is that the influence of configuration or conformation of the transport probe on transglomerular transport still remains to be established.

Influence of charge

The relatively low fractional clearance of albumin has also been discussed in terms of the negative charge of the molecule (valence $Z = -12$ to -18, Table 3). If this charge interacts electrostatically with the fixed negative charge of the GBM matrix and other charges of the glomerular unit, then it is generally thought that repulsion would occur leading to decreased transglomerular transport.

Efforts at demonstrating this charge effect by chemically modifying the net charge on the protein again have not given consistent results (Table 4). The most detailed studies on the influence of protein charge on fractional clearance are by Rennke et al [32, 33] where it was established that there is a significant decrease (by a factor of 1/8.7) in the clearance of negatively charged HRP material ($Z = -11$; pI < 4.0) as compared to neutral HRP (pI 7.3 to 7.5). The cationic HRP (8.4 to 9.2) had the highest clearance, but because cationic material will bind to the GCW it is difficult to interpret, unambiguously, the meaning of clearance measurements with such materials [34]. Recent studies also have demonstrated that analysis of HRP by enzyme activity, as was performed in these studies, severely underestimates the level HRP, particularly for the anionic material as it appears to be degraded during filtration [35]. The actual differences between neutral and anionic material are probably less than a factor of 3. A similar difference is seen for anionic and neutral amylase [36]. On the other hand, Purtell et al [14] found no difference between rat or human native albumin with neutral albumin, but did demonstrate an increased fractional clearance of cationic albumin. The studies of Bertolatus et al [16] also suggest that anionic and neutral IgG were similar, although inconsistent results have been obtained [13] (see also [37]). Surprisingly, they found that the clearances of high molecular weight IgG and ceruloplasmin were not that different to albumin (Table 4). Other studies comparing the clearances of neutral IgG (pI 5.8 to 7.3) and anionic IgG4 (pI 5.5 to 6.0) have yielded particularly inconsistent results: the IgG/IgG4 fractional clearance ratio in humans has been found to be 1.35 [38], 2.43 [23] and ~10.0 [37]. At present the major feature of these studies is that: (i) cationised proteins always seem to be transported faster than their neutral or anionic counterparts; (ii) neutral proteins have significantly lower clearances than dextran of equivalent hydrodynamic size (HRP 0.062; dextran 0.4: neutral albumin 0.006; dextran 0.19); and (iii) while inconsistent results have been obtained in comparing anionic and neutral protein species, generally the fractional clearance of neutral proteins is only two to three times that of their negatively charged counterparts.

Other factors which appear to influence albumin permeability, by mechanisms that are unclear at present, are the presence of non-albumin plasma proteins such as orosomucoid [39] and possibly the concentration dependent albumin interaction with the capillary wall as studied in other capillary beds [40, 41].

Fractional clearance of charged dextrans

The most compelling evidence to support the charge selective nature of the glomerular filter has been associated with the series of *in vivo* studies of the fractional clearance of charged dextran

polysaccharides. The influence of the GBM and the filter as a whole is thought to be quite specific as these molecules apparently do not undergo tubular reabsorption.

Negatively charged dextrans, like dextran sulfate, have a significant restriction in transglomerular transport as compared to uncharged dextran, whereas positively charged dextrans (DEAE dextran) have facilitated clearance for a given hydrodynamic radius (Fig. 1). In keeping with the evidence which would suggest that the negative charge of albumin is responsible for its lowered fractional clearance, it has been argued that results with dextran sulfate [albeit with higher valence (~36 Å, Z = ~−60)] would support this. Dextran sulfate clearances in rats for molecular radius of 30 Å have been shown to vary from 0.006 to 0.2 and for radius of 40 Å from 0.0004 to 0.026 [43]. In general the values are higher than that for albumin.

Whether the enhanced clearance of the cationic species, such as DEAE dextran, is strictly a function of charge is not yet settled. Some have argued that some cationic macromolecules may bind to anionic sites and damage the GCW to make it leakier which, in turn, yields higher fractional clearance. Our own studies of the fractional clearance of DEAE dextran in the isolated perfused rat kidney demonstrated that there indeed was significant binding of DEAE dextran to the glomerulus and this was accompanied by a reduced fractional clearance as compared to dextran measured in the same system [34].

Donnan partitioning—An explanation of the charge effects

The anionic sites of the GCW and particularly the GBM are believed to be responsible for charge selectivity [1, 32, 42, 45]. The electrostatic basis of charge selectivity was considered to be very much substantiated by the Deen-Satvat-Jamieson model [46] that used the classical Nernst-Planck equations, where it was assumed that the GBM was like an anion exchange membrane and that mobile charged probes would undergo electrostatic interaction within it. The theoretical, biophysical interpretation of renal charge selectivity by Deen, Satvat and Jamieson [46] has been based on the fact that transglomerular macromolecular transport occurs as a passive process in the intercellular space of the capillary wall and across the GBM. The fixed negative charges of the basement membrane then account for transport charge selectivity. The theory takes into account two processes: (i) the partitioning of the macromolecule at the capillary-GBM interface due to charge interaction (Donnan partitioning); and (ii) the transport at the charge probe within the electrochemical environment of the GBM. Major charge effects are envisaged only to occur with the Donnan partitioning, as transport (whether convective or diffusive) within the GBM is not considered to manifest the marked differences due to charge; this has been confirmed experimentally [47]. The Donnan partitioning has then been used to evaluate an effective charge concentration of the GBM on the basis of the fractional clearance data. Values of the effective charge of the GBM were estimated to be in the range of 100 to 170 mEq/liter (an equivalent is a mole of charge or mole of associated counterion like Na^+) with one estimate at 60 mEq/liter (reviewed by Maddox, Deen and Brenner [43]). An anion charge of 165 mEq/liter is equivalent to 40 to 45 mg/ml heparan sulfate.

Perhaps the major criticism of this approach is that partitioning of charged macromolecules with charged membranes due to Donnan has been experimentally tested and shown not to hold [47]. Further, the large partition coefficients required do not fit with the thermodynamic properties of charged molecules in question [47].

Anion charge of the glomerular capillary wall

Apart from concerns regarding the assumptions associated with the DSJ model experimental estimates of the GBM anion charge have been demonstrated to be generally an order of magnitude lower. Bray and Robinson [48] titrated the isolated GBM to obtain a negative charge of 12 mEq/liter. They suggested that this was at least twice as much as the value obtained from estimating the charge knowing that heparan sulfate only accounts for 1% of the dry weight of the rat GBM [49, 50]. Estimates of GBM charge through a $^{22}Na^+$ and $^{36}Cl^-$ exchange technique gave values of 7.6 ± 0.4 mEq/liter [51]. It could be argued that the low values of GBM charge are the result of losses of heparan sulfate, particularly as a cell surface component, during the course of GBM purification. Further, it is noted that the above estimates are only average GBM quantities and that higher concentrations result from specific localization of the material such as in the lamina rara. Other measurements of NaCl and water transport across the GWC, as described below, also suggest a low charge concentration.

In another type of capillary, namely single microvessels of frog, the charge selectivity of two globular proteins (lactalbumin and ribonuclease) was analyzed with the use of a Donnan-type model for electrostatic partitioning which gave a charge density as 11.4 mEq/liter [52].

Donnan osmotic pressure

Wolgast and Öjteg [53] correctly recognized that the GBM is not rigid but deformable [54, 55] and has an internal osmotic pressure. In consideration of Donnan osmotic pressures on the basis of very small differences in ion distributions, which may be subject to error, they arrived at a GBM charge of ~30 mEq/liter.

Transglomerular transport of NaCl

Charged membranes are known to restrict the transport of simple electrolytes like NaCl. Yet with the ratio of chloride in the Bowman's space/plasma equal to one [43] it is clear that the GBM does not offer any significant restriction to NaCl transfer. Theoretical and experimental studies suggest that a GBM charge of less than 200 mEq/liter exert only minor effects on electrolyte transport [27].

Transglomerular transport of water

While the transglomerular transport of NaCl is not sensitive to heparan sulfate-NaCl interaction, studies have established that the transport of water is sensitive to heparan sulfate-water interaction. This interaction essentially takes the form of a surface area to volume obstruction effect of the flow of water over the glycosaminoglycan chain. Therefore, water transport through the GCW as reflected in the GFR may be determined by the GBM heparan sulfate content.

The value of the hydraulic conductivity, as measured by sedimentation velocity analysis, of heparin-like polysaccharides has been measured as a function of concentration [47]. Its value decreases with increasing concentration (as expected) and is independent of ionic strength of solvent and degree of sulfation of the polysaccharide. The studies demonstrate that the hydraulic conductivity of all heparin-like polysaccharides is determined by

the nature of the glycosidic linkage [56]. If we assume that the single nephron GFR is governed essentially by the heparin-like polysaccharide concentration, then an estimate of its concentration can be made. The volume flux of solvent (J_v) defining the glomerular filtration rate (GFR) as a function of net pressure (ΔP) and the ultrafiltration coefficient of a single nephron (K_f) is

$$J_v = K_f \Delta P$$

where

$$K_f = k/Al\eta$$

and where k is the hydraulic conductivity; A is the effective filtration area; l, thickness of regions containing heparan sulfate; and η, is solvent viscosity.

The hydraulic conductivity (k) can then be calculated using K_f for the single rat nephron as 0.1 nliter/second per mm Hg [57, 58]. This will be a minimal value as this does not take into account osmotic gradients that may be generated within the GBM under a pressure gradient [59]. The effective filtration area (A), determined by endothelial cell fenestrae is taken as 10% [46] of the total nephron surface area of 0.0019 cm^2 [60]. Heparan sulfate appears to be located specifically in the laminal rara regions [3, 4, 8]. Assuming that each lamina rara region is 50 nm (for rat GBM) and they act in series we assign a value of 100 nm for l.

Using these quantities in equation gives a k of 2.76×10^{-14} cm^2 which corresponds to a heparin-like polysaccharide concentration of 40 to 45 mg/ml [47] or 165 mEq/liter, as estimated by Deen et al [46]. It is emphasized that this will be an overestimation of GBM charge concentration as no account has been made for the flow resistance effects of GBM collagen on the GFR.

Specific analysis of the variation of GFR with the removal of heparan sulfate has not been investigated, though it should be noted that neutralization of the heparan sulfate would be expected not to significantly alter the GFR. However, in a study where charge selectivity was destroyed through removal of colloidal-iron staining material (presumably heparan sulfate) in rats with nephrotic serum nephritis [42] the single nephron GFR was shown not to increase as predicted *vide supra,* but actually decreased. Similar changes have also been noted for severe reductions in renal mass [19]. These results would suggest that actual GBM heparan sulfate concentration is too low to affect GFR, that is, <10 mg/ml or 40 mEq/liter.

Isolated GBM preparations have considerably higher ultrafiltration coefficient, of the order of 1 nl/second per mm Hg [54, 61], which demonstrate that their heparan sulfate content is relatively low and that other components of the GCW may govern hydraulic conductivity, such as the slit processes. Similar high ultrafiltration coefficients have been obtained in acellular glomeruli [62].

Evidence for electrostatic charge influence on transport

Neutralization of GBM charge

The rationale behind these studies is that the addition of exogenous polycation to neutralize the fixed anion charge of the glomerular wall may perturb transglomerular transport of the probe in some way [14, 32, 33, 44, 63–66]. Oddly, the degree of neutralization has never been measured. In any case, polycationic macromolecules have diverse effects upon permeability. The possible effects include: (i) the distortion of the GBM gel network thereby altering porosity; (ii) altered glomerular cell metabolism even to extreme toxic effects; (iii) varying degrees of proteinuria; (iv) conversion of negative sites to positive sites through an imbalance of binding (charge reversal). These various manifestations of polycation neutralization have not been clearly delineated.

In an attempt to decrease electrostatic interaction of circulating proteins with glomerular charge the filtration properties of isolated kidneys were studied in buffers with molarities up to 2.5 M [67]. This resulted in increased amounts of native ferritin in the GBM as determined by ultrastructural analysis and a decreased permeability to insulin (by 1/3) and inulin (by 1/20). It was concluded that the GBM was clogged at these high molarity buffer and that the sulfated glycosaminoglycans serve as anticlogging agents, although no evidence was provided that albumin accumulated at the GCW. This is in spite of the fact that no changes were observed at 1.5 M buffer molarity for insulin transport and 0.5 M buffer molarity for inulin transport, which represent ionic strengths more than enough to significantly mask any electrostatic interaction between proteins and sulphated glycosaminoglycans. These studies with circulating polycations do reinforce the fact, however, that glomerular charge and ultrastructure are intimately linked in some way.

Influence of heparan sulfate

There has only been indirect experimental evidence to suggest that GBM heparan sulfate participates in influencing the electrostatic environment of charged transport probes in transit. Rosenzweig and Kanwar [68] have employed specific glycosaminoglycan (GAG)-degrading enzymes in perfused kidneys to examine the distribution of [^{125}I]albumin between the urinary space and capillary by quantitating light microscopic autoradiograms. The capillary grain/urinary grain ratio decreased by 47% when hyaluronan was digested, by 54% when hyaluronan and chondroitin sulfate were digested, and by 79% when hyaluronan, chondroitin sulfate and heparan sulfate were digested. The surprising feature of these results is that the digestion of minor glomerular GAG components, namely hyaluronan and chondroitin sulfate had such a profound influence of the permeability to albumin. Specific removal of the heparan sulfate GAG was not investigated. However, a previous study [69] demonstrated that heparinase digestion removed all GBM binding sites to cationic ferritin and that this heparinase treatment (not hyaluronidase or chondroitinase ABC) leads to the appearance of native ferritin (radius = 61 Å) in the urine.

In a more recent study, Adal et al [70] have demonstrated that the binding of albumin to glomeruli and the GBM is enhanced when these tissues are subject to heparinase digestion. The enhancement of albumin transport in situations where there is a loss of GCW heparan sulfate might be explained by weak interactions between albumin and GCW components which would tend to elevate albumin concentrations in membrane pores.

Many other studies have established a correlation of a change in the GBM levels of heparan sulfate with increased glomerular permeability particularly in disease states, although the mechanism of these effects is unclear. Of recent interest is that exogenous GAGs may prevent albuminuria in diabetic rats [71], and this may be a metabolic effect as it is known that exogenous sulfated polysaccharides may stimulate the synthesis of heparan sulfate in endothelial cells [72].

Monoclonal antibodies directed against the heparan sulfate GAG chain in the GBM [73] are known to induce proteinuria. While neutralization of the charge is unlikely, conformation and structural integrity of the GBM in the GCW appears to be an important factor demonstrated here in determining transglomerular transport. It had been noted earlier that polyclonal antibodies directed against the protein core of the GBM heparan sulfate proteoglycan also caused proteinuria [74–76].

The conclusion from these studies is that GBM heparan sulfate functions not as a charge barrier but acts to maintain the structural integrity of the glomerular filter and particularly those pore structures that determine size selectivity. It also appears to maintain protection of those sites in the GCW that may bind albumin.

Glomerular permselectivity is not mimicked in isolated glomerular basement membrane systems

These studies have used isolated glomerular basement membrane strips compacted into a composite layer on a porous structural support [77, 78]. Both hydraulic and macromolecular permeabilities of these layers are much greater than that reported for the intact GBM [54, 61]. The rejection of albumin by these layers has been shown to be quite complex. It turns out that it is dependent on stirring; with no stirring, there is a build up of albumin next to the membrane (concentration polarization) and the rejection of albumin falls appreciably [77], possibly due to an osmotically induced change in the membrane structure due to the adjacent albumin layer. Also for these layers, there is little difference in the permeability of dextran and dextran sulfate over the range of radii of 15 to 42 Å. There was, however, a significantly increased permeability of DEAE dextran over the same size range. A low degree of charge selectivity has also been observed with different charged albumins [55] which would also argue against any specific role of carboxyl groups retained in GBM preparations on charge selectivity *in vivo* [79]. The relative difference in permeability between albumin and dextran (dextran rejection 0.9 to 0.95, albumin rejection 0.96) did not appear to reflect that seen *in vivo* [48, 54].

A feature of the isolated membranes that has not been examined for intact systems is the recognition that pressure-flux relationships are nonlinear [78], which demonstrates that the membrane is compressible [54–56].

Morphological studies

A large body of work has been carried out since the 1960's in the renal field in the pursuit of the ultrastructural location of tracer macromolecules. Much of the aim of this sort of exercise is to identify regions within the glomerular filter (essentially extracellular ones) where restriction of the transport probe may occur. These studies have been reviewed elsewhere and will not be considered here. A good deal of caution should accompany the interpretation of these types of results particularly when conclusions concerning transglomerular transport are made. There is no *a priori* relationship between localization of the probe and fractional clearance. The ultrastructural localization is performed under nonequilibrium conditions whereas fractional clearance is a steady state transport. Localization may mean a genuine transport restriction but then it may also represent a binding interaction. Further, the residence of the exogenous probe may exert cooperative effects to influence further localization through changes in filter structure. Overall, it is very difficult to interpret the ultrastructural localization data alone in terms of transglomerular transport.

Hemodynamic effects

Transglomerular transport of macromolecules may be influenced by factors other than cell uptake, pore size and charge on the GCW. These are hemodynamic factors that include blood flow, the mean transcapillary hydraulic pressure difference, the plasma protein concentration and the capillary ultrafiltration coefficient. Theoretical relationships of the influence of these hemodynamic factors on fractional clearance have been derived [80] but have yet to be fully tested. Chang et al [12] were able to increase the dextran fractional clearance by a relatively small amount by increasing the plasma flow rate that was in good quantitative agreement with the theory. Ryan and Karnovsky [81] showed that cessation of glomerular flows and pressures may cause (reversibly) increased permeability of the GCW. However, these results are controversial as other studies have shown no effect on lowering renal plasma flow [82, 83]. There have been no reasonable explanations for these effects. But what is clear is that the integrity of the GWC appears important particularly through its interaction with albumin [39–41, 48, 54, 77] under different conditions. In all these studies there has not been the suggestion that hemodynamic factors could explain the size and charge selectivity profiles discussed *vide supra*.

The issues of the modes of macromolecular transglomerular transport have been discussed in terms of convective transport or diffusive transport yet there are no established conclusions about how this may affect the fractional clearance data. Certainly for small molecules, like inulin, the dominant transport is expected to be convection as the transglomerular concentration gradients would be too small to drive any significant diffusion.

Cell-mediated transport

Cell-mediated processing of proteins exposed to the glomerular vascular bed has not been considered to be significant. Rather, it is generally viewed that transport will be extracellular in this relatively highly fenestrated region. Renkin and Gilmore [11] state "the role of vesicular transport in glomerular filtration must be entirely negligible. First, endothelial fenestrae provide an alternate route of lower resistance; second, anything transported by the vesicles is exposed to the sieving action of the basement membrane and epithelial slits. The epithelial vesicles are probably related to reabsorption of protein from the outer face of the basement membrane and from Bowman's capsule."

In a review article Simionescu and Simionescu [84] have described various aspects of endothelial cell-mediated processing of proteins in non glomerular vascular beds. Endothelial cell processing of probes, commonly used in glomerular transport studies, are candidates for cell uptake in other capillary beds. This may take the form of fluid phase endocytosis (horseradish peroxidase, native ferritin together with dextran), nonspecific adsorptive endocytosis (native ferritin), and specific endocytosis and trancytosis (insulin, albumin). Albumin binding proteins function in specific receptor-mediated binding and trancytosis of albumin across cultured aortic endothelial cells [85]. It is clear that the contribution of any cell-mediated processing in glomerular endothelial cells has yet to be fully examined.

The potential importance of the epithelial cell in governing glomerular permeability of proteins is suggested by its intracellular machinery characteristic of endocytosis. The protein uptake by epithelial cells may be a contributing factor to the unusually low Bowman's space to plasma ratio of filtered protein. There is simply a paucity of studies associated with epithelial uptake of transport probes. Earlier studies with ferritin and dextran uptake in the epithelium had been studied ultrastructurally [82, 86, 87].

Like the endothelial cell, the likelihood of the mesangial cell taking a direct part in protein filtration would be low owing to the relatively small fraction of the filtration surface that it occupies.

New evidence of dextran sulfate transglomerular transport

It has always been assumed that the dextran sulfate used to study glomerular charge selectivity was inert, that is, it was not taken up by any glomerular cells and changed chemically. We have now established this 'assumption' is incorrect. During charge selectivity clearance of dextran sulfate, it has been established that there is a considerable steady state level of glomerular dextran sulfate which has a relatively short half-life of glomerular residence (~2 to 4 min). The half life can be extended to more than one hour by cycloheximide [88]. The specific localization of [^{125}I]dextran sulfate in glomeruli has been demonstrated [89] where dextran sulfate binding is predominantly to cellular elements rather than the GBM [90]. Preglomerular intracellular vesicles have been isolated post perfusion and have been shown to contain dextran sulfate [89]. Additionally, analysis of dextran sulfate fraction from the glomerulus and urine has also established that significant desulfation of the probe had occurred [91]. These results demonstrate unequivocally that dextran sulfate is taken up by glomerular cells, most probably endothelial cells, by a receptor-mediated mechanism. When the receptors are recycled they release the desulfated dextran sulfate ligands which may then be transported through the GBM. Studies have demonstrated that intravenously administered dextran sulfate in rats rapidly yields plasma samples that are considerably desulfated. Therefore, when using this probe, it will be presented to the kidney as a mixture of sulfated, partially desulfated and completely desulfated products.

The concept of a cell-mediated mechanism associated with the processing of dextran sulfate raises the question of saturating cell uptake. The studies described above for the isolated perfused kidney were with dextran sulfate at 15 μg/ml in the perfusate and were not at saturating levels of glomerular uptake. Studies *in vivo* were performed with a bolus injection to give an average plasma concentration of 80 μg/ml. Other studies on the clearance of dextran sulfate have not described the plasma concentrations of the probe, although in the studies of Chang et al [12] they describe the plasma concentration of dextran to be <100 μg/ml, and subsequent studies on dextran sulfate appeared to be performed under similar conditions. If receptor-mediated uptake of dextran sulfate governs the behavior of most of the dextran sulfate then its fractional clearance should be concentration dependent, and charge selectivity should be negligible at high dextran sulfate concentrations. The concentration dependence of the fractional clearance of dextran sulfate has been demonstrated in the isolated perfused kidney [92]. The dextran sulfate concentration in the perfusate was studied over a range of 4 μg/ml to 1000 μg/ml. The fractional clearance of the dextran sulfate was lowest for the perfusate concentration of 4 μg/ml. The fractional clearance increased in value as the perfusate concentration was increased up to 200 μg/ml dextran sulfate. The clearance of dextran sulfate did not change over the concentration range of 200 to 1000 μg/ml of dextran sulfate. Dextran clearance studied in the presence of either 200 μg/ml or 1000 μg/ml dextran sulfate was not significantly different when compared over a wide range of radii to dextran sulfate clearance when studied at relatively high concentration [92]. These results demonstrate that charge selectivity is saturable above 200 μg/ml dextran sulfate and markedly concentration dependent below this concentration. Size selectivity as measured by dextran clearance was unaffected over the range of dextran sulfate concentrations studied. Dextran sulfate, when used at relatively high concentrations but relatively low in relation to the charge content of the GBM (1000 μg/ml dextran sulfate corresponds to a charge concentration of approximately 3 mEq/liter, whereas the fixed anion charge content of the GBM is 7.6 mEq/liter [51]), undergoes transglomerular transport in the same manner as the uncharged dextran.

The apparent reduction of the fractional clearance of dextran sulfate, when used at relatively low concentrations, as compared to dextran (which is not taken up by glomerular cells) may result from two factors: (1) the lowering of the plasma dextran sulfate concentration at the GBM/capillary interface due to uptake by neighboring endothelial cells; and (2) the lower molecular weight and size of the desulfated material on the gel chromatographic column used for fractional clearance analysis.

Other evidence to support glomerular cell uptake of the sulfated polysaccharides

Desulfation of other exogenous, intravenously-administered sulfated polysaccharides has been observed including pentosan polysulfate [93], heparan sulfate [94], and dermatan sulfate [95]. The sites of desulfation have not been established but they are likely to occur in the liver and kidney.

Studies of dextran sulfate clearance in *ex situ* kidney perfusions have demonstrated that the material that appears in the urine is partially desulfated and that sulfatase activity exists in both glomerular and tubular extracts. The fact that glomerular endothelial cells may actively take up dextran sulfate is not unusual as endothelial cells in other vascular beds have been shown to endocytose a variety of sulfated polysaccharides [96, 97]. Human vascular endothelial cells have been shown to catabolize exogenous glycosaminoglycans, including heparin [94, 98]. The binding of heparin is saturable through at least two binding sites. These sites are not specific for heparin as other sulfated polysaccharides may bind. A high proportion of the bound heparin is ultimately endocytozed and degraded [98, 99]. Intracellular uptake of heparin by endothelial cells may also result in its protracted release [72] and prevent morphological renal alterations and albuminuria in diabetic rats [71]. Heparin and dextran sulfate uptake by cultured endothelial cells is known to stimulate the synthesis of heparan sulfate [100]. Liver endothelial cells have been demonstrated to bind and rapidly endocytose (within minutes) chondroitin sulfate and the unsulfated anionic polysaccharide, hyaluronan [101–103]. The receptors responsible for the internalization of hyaluronan are recycled back to the cell surface very rapidly during the continuous endocytosis of the macromolecule, and this recycling is reduced significantly (by 50%) in the presence of cycloheximide [103].

The role of glomerular endothelial cell in the transport of sulfated polysaccharides is confirmed, but there is a paucity of

information regarding other glomerular cells and their interaction with sulfated polysaccharides. It is well known that accumulation of macromolecules in the mesangium may occur [87], particularly in disease states [104, 105].

Concluding remarks

Various factors, including charge effects, have been studied to explain why albumin has a lower fractional clearance than dextran and other uncharged random coil molecules of equivalent hydrodynamic size. These factors are reviewed in this article particularly in relation to the role of the molecular and cellular elements of the glomerular capillary wall in governing transport. A comparison of the fractional clearance of the spherical Ficoll to dextran revealed that spherical molecules may have lower clearance and this may account, in part, for the albumin transport when considered on size alone. Unfortunately, there has been considerable variation in the published quantitative fractional clearance values of the various probes concerned and clearly, further comparative studies should include estimates of different probes on the same system and their hydrodynamic size measurements performed under conditions that they would encounter during filtration.

Is there charge selectivity associated with glomerular ultrafiltration?

The answer to this question is that some transport probes do manifest differential transport based on their charge, but it is likely that the influence of electrostatic interactions with the negative charge of the glomerular capillary wall will not be as significant as originally thought. The apparent charge selectivity in the dextran sulfate/dextran system is influenced by glomerular cell-mediated interactions with dextran sulfate and biochemical changes to this probe. Intact glomeruli are required as isolated GBM preparations do not show charge selectivity. Further, it is difficult to support the explanations of the relative differences between the two probes on the basis of the charge content of the GBM and physicochemical models of the electrostatic interaction. Some protein systems, particularly as studied for different charged derivatives of the same protein, manifest charge selectivity although there is a disconcerting lack of consistency between published data. The morphological evidence for charge barriers has been qualitative and not of value in relation to the steady state clearance measurements. While there may be an electrostatic basis for some charge selectivity being exerted on charged proteins it is apparent that an unambiguous interpretation will only come from a complete quantitative analysis of both the glomerular binding and cell uptake, as well as tubular processing.

It is apparent from these studies that there is still no definitive answer to the vital question as to why the glomerular capillary wall has a relatively low permeability to albumin. Further studies are required to address this important issue if there is to be correct understanding of the structure and function of GCW components in governing transglomerular transport in health and disease.

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