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Size-selectivity of the glomerular barrier to high molecular weight proteins: Upper size limitations of shunt pathways

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Size-selectivity of the glomerular barrier to high molecular weight proteins: Upper size limitations of shunt pathways. To evaluate the large pore radius of the glomerular capillary filter, plasma-to-urine fractional clearances of a number of endogenous proteins were assessed in normal and in nephrotic Wistar rats in which proximal tubular reabsorption had been inhibited using lysine. The proteins studied varied in radius from 16.2 Å (β_2-microglobulin) to 90 Å (α_2-macroglobulin). The nephrotic syndrome was induced by puromycin aminonucleoside (PAN). A marked restriction of the transport of large proteins across the glomerular capillary wall was found, indicating that there are no non-discriminatory 'shunt pathways' in the glomerular barrier. Rather, there seems to be large pores of radius 110 to 115 Å accounting for the clearance of large proteins into the primary urine. This protein excretion pattern was almost the same for control and nephrotic rats, except that in the latter, the number of large pores was increased 170 times. The ratio between the number of large and small pores was calculated to be $\approx 7 \cdot 10^{-7}$ in normal rats and to $1.3 \cdot 10^{-4}$ in PAN nephrotic rats, assuming no classic shunt pathways. If classic shunt pathways had still existed, they would normally contribute to no more than $\approx 10^{-5}$ of the total glomerular filtration rate. We postulate that very large macromolecules like IgM will not pass the glomerular filter at all under normal conditions, whereas the urine concentration of α_2-macroglobulin will normally be extremely low.

The passage of solutes from blood to interstitium in the body can be adequately described by a two-pore theory of capillary permeability [1–3]. This theory predicts the transcapillary passage of small solutes and water to occur mainly through equivalent 'small pores' of a radius 40 to 50 Å or negatively charged pores or slits of radius ≈60 Å. The passage of albumin is severely restricted across these pores. Albumin, and even larger macromolecules, can be modelled to pass the capillary membrane through a very small number of 'large pores' or 'shunt pathways' having a radius of approximately 250 Å. The two-pore theory is based on measurements of steady-state fractional clearances of proteins from plasma to interstitium or lymph.

The measurement of fractional clearances (θ) of proteins across the glomerular capillary wall to the primary urine presents formidable problems, because a vast majority of the proteins appearing in the Bowman's capsule ultrafiltrate are reabsorbed in the proximal tubules before reaching the final urine. In humans, the urinary fractional clearance of albumin is thus of the order of 2 to $6 \cdot 10^{-6}$, whereas the corresponding fractional clearance to the *primary* urine is thought to be of the order of $1 \cdot 10^{-4}$ or slightly less. In rats, the corresponding numbers may be as high as 1 to $2 \cdot 10^{-4}$ and 1 to $2 \cdot 10^{-3}$, respectively [4, 5].

Due to the presence of proximal tubular reabsorption, endogenous proteins have only rarely been chosen to assess glomerular permselectivity, except in micropuncture studies [6, 7]. However, the latter are usually complicated by the adsorption of proteins to the sampling pipette or by active reabsorption of proteins in nephron segments proximal to the pipette. Other ways of overcoming the problems caused by tubular reabsorption have been based on inhibition of the reabsorption in, for example, isolated kidneys, by tissue cooling to 8°C [4, 5] or using inhibitors of tubular reabsorption such as lysine, maleate or cytochalasin B [8–10].

In a majority of previous studies, dextrans (negatively charged or uncharged) have been used to probe the size-selectivity of the glomerular filter, since dextrans are neither actively reabsorbed nor secreted. Such measurements were recently extensively reviewed [11]. Using uncharged dextrans, the 'small pore' radius in the glomerular filter has been determined to be ≈50 to 55 Å both in the human and rat. To explain the passage of macromolecules larger than albumin across the glomerular barrier a 'shunt pathway' accounting for 0.1 to $1 \cdot 10^{-3}$ of the total ultrafiltration coefficient has been invoked [11, 12]. Negative charges in the 50 Å pores were assumed to be mainly responsible for the prevention of albumin from leaking across the glomerular wall.

Dextran molecules are, however, long-chained flexible molecules and new data using neutral cyclic molecules as probes for glomerular permeability have indicated that the small-pore radius may be rather of the order of 30 Å than 50 Å. Thus, the major portion of albumin restriction seems to derive from size-selectivity rather than from charge-selectivity of the glomerular capillary wall [13]. Indeed, the charge-selectivity for anionic and neutral horseradish peroxidase was recently shown to be of the same order of magnitude as that in peripheral capillaries. Thus, the glomerular capillary wall anionic charge-barrier seems to have reduced the clearance of negatively charged horseradish peroxidase only by a factor 2 to 3 [14, 15].

The present study was performed in order to evaluate the size of what has previously been known as 'shunt pathways' of glomerular permselectivity. If such non-discriminatory pathways

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exist, then the fractional clearance of all proteins larger than a certain large solute size would be about equal, which was tested in the present study. To inhibit tubular reabsorption of large proteins, we used lysine inhibition [10] in normal rats and in rats with puromycin aminonucleoside (PAN)-induced nephrosis. The investigated proteins were represented by β_2-microglobulin (mol radius 16.2 Å), orosomucoid (mol radius 29 Å), albumin (mol radius 36 Å), IgG_2a (mol radius 55 Å) and α_2-macroglobulin (mol radius ≈90 Å). We were surprised to find a marked restriction of large proteins across the glomerular capillary wall both during normal conditions and in PAN nephrosis. Thus, if still existing, classic shunt-pathways should contribute only at maximum $\approx 10^{-5}$ of the entire glomerular filtration rate (GFR).

METHODS

Twenty-six female Wistar rats (Møllegard, Copenhagen, Denmark), weighing 185 to 270 g were divided into two groups. One group, containing 10 animals, was given a single intraperitoneal injection of PAN at 15 mg/100 g body wt (Sigma Chemical Co., St. Louis, MO, USA). The drug was dissolved in one ml of 9 g/1000 ml sodium chloride [16–18]. No apparent acute toxic effects of drug infusion were noted (see below). The remaining 16 animals were left untreated. All the animals were allowed free access to food and water. In the PAN treated group the experiments were performed when the urinary protein, screened by urinalysis strips for protein (Albustix®; Miles Inc., Ekhart, IN, USA), showed 4+, which occurred on day 9 to 11 after the injection of PAN. The controls were used after 9 to 11 days.

Seven animals in the PAN group and 13 animals in the control group were used in the experiments comprising measurements of albumin, IgG_2a, orosomucoid and β_2-microglobulin. The remaining three rats in each group were used in the experiments comprising assessments of α_2-macroglobulin.

All the animals were investigated according to the subsequent protocol. Following induction of sodium pentobarbital anesthesia (30 mg/kg body wt) the rats were placed on a temperature-regulated table, which maintained rectal temperature at 37°C. The blood vessels were cannulated using polyethylene tubing (PE-50): the left internal jugular vein for infusing compounds, the left carotid artery for obtaining blood samples and the tail artery for continuous monitoring of blood pressure on a polygraph (Grass model 7B; Grass Instrument Co., Quincy, MA, USA). Urinary bladder was catheterized via the urethra using small tubing (PE-50). After completion of the surgical preparation, which required one to one and a half hours, the remainder of the experiment lasted approximately four hours.

Experiments were initiated by a single injection of 50 μl of ^{51}Cr-EDTA (10 mBq/ml; Amersham Radiochemical Centre, Ltd, UK) dissolved in 0.3 ml saline. The injection was followed by infusion at 100 μl ^{51}Cr-EDTA (in 6 ml saline) 10 mBq/ml per hour. After 60 minutes the infusion was supplemented by infusion of L-lysine monohydrochloride (221 mmol/liter, 384 mOsm/liter; Sigma), at 55 μmol/kg/min. The infusion was made by preparing a solution of lysine (free base) in distilled water and adjusting the pH to 7.4 with 1 N HCl acid. ^{51}Cr-EDTA was given at the same rate as before.

The dosage of lysine was calculated from pilot studies and from literature [19–22]. Higher doses caused renal failure, since lysine has been shown to be nephrotoxic [23]. Also, when the fluid support to animals has been reduced, oliguria has usually occurred after lysine administration [23].

An additional two rats were investigated according to the study protocol in order to investigate the ultrastructural status of glomerular and tubular epithelium after lysine infusion. The animals were killed by intravenous injection of potasium-chloride, whereupon the kidneys were dissected out and cut in slices, which were immersion-fixed in a solution of 4% of buffered formaldehyde, and further processed for parafin embedding. Sections were cut at a thickness of 4 μm, which were stained in hematoxylin and eosin, Masson Trichrome alum, silver methenamine, Van Gieson, picrosirius and Mac Manus.

Duplicate 10 μl samples of arterial blood were taken every 30 minutes for radioactivity measurements in vials prefilled with 0.5 ml of 0.9% sodium chloride. Samples (50 μl) for hematocrit were collected every 60 minutes. Blood (0.5 ml) was drawn from the arterial line at the end of each experiment for measurement of protein concentrations. The blood samples were centrifuged for 12 minutes at 5000 r/min and the plasma was separated. Urine was collected at 30 minute intervals from the urethra.

The fractional plasma clearance (θ) was computed by the formula:

$$\theta = \frac{C_u \cdot V_u}{\bar{C}_p \cdot GFR}$$

and GFR was calculated from:

$$GFR = \frac{C_{u_E} \cdot V_u}{C_{pw}}$$

where the plasma water concentration of CrEDTA (C_{pw}) was obtained from:

$$C_{pw} = \frac{C_{p_E}}{0.984 - 0.000718 \cdot C_{prot}}$$

where C_{u_p} and C_{u_E} are urinary concentration of protein and CrEDTA, respectively, V_u represents urine flow per minute, C_{prot} and C_{p_E} represents plasma concentration of protein and CrEDTA, respectively, GFR was determined as the ^{51}Cr-EDTA plasma-to-urine clearance, and $\bar{C}_p$ (average plasma protein concentration in glomeruli) was calculated by the formula:

$$\bar{C}_p = \frac{C_p + C_p \cdot \frac{1}{1 - FF \cdot (1 - \theta_i)}}{2}$$

where C_p is protein content in plasma entering the glomeruli, $C_p \cdot 1/(1 - FF \cdot (1 - \theta_i))$ is the plasma protein concentration in efferent plasma and θ_i is the sieving coefficient for proteins in plasma before correction for the plasma concentration process in glomeruli.

Determination of protein concentrations

The plasma and urine concentrations of albumin, IgG_2a and orosomucoid were determined by single radial immunodiffusion [24] using, for albumin and IgG_2a, monospecific rabbit anti-rat antisera (Kemila, Sollentuna, Sweden), and for orosomucoid a specially prepared rabbit anti-rat antisera (Agri Sera, Vännäs, Sweden). Eight microliters of undiluted plasma or urine were

Table 1. Glomerular filtration rate (measured as ^{51}Cr-EDTA plasma clearance) and plasma concentrations of β_2-microglobulin, albumin, orosomucoid and IgG_2a in 16 control rats and in 10 rats with puromycin aminonucleoside (PAN) induced nephrosis

	Control rats median (*range*)	PAN nephrosis rats median (*range*)
Plasma clearance of ^{51}Cr-EDTA	1400 (1007–1868)	705 (314–917)
Plasma β_2-microglobulin *mg/liter*	4.6 (3.3–8.9)	1.9 (1.5–2.5)
Plasma albumin *mg/liter*	22020 (16320–25200)	4937 (3200–6720)
Plasma orosomucoid *mg/liter*	115 (69–216)	14 (7–22)
Plasma IgG_2a *mg/liter*	1156 (880–1566)	66 (26–98)

Table 2. Medians (ranges) of fractional plasma-to-urine clearances for β_2-microglobulin, orosomucoid, albumin, IgG_2a and α_2-macroglobulin in control rats before and after treatment with lysine

Protein	Before lysine treatment	Lysine treated rats
β_2-Microglobulin	2.89^{-4} (1.21^{-4}–9.05^{-4})	1.30^{-1} (7.18^{-2}–2.47^{-1})
Orosomucoid	1.67^{-4} (6.16^{-5}–5.89^{-4})	3.40^{-4} (1.33^{-4}–1.29^{-3})
Albumin	5.39^{-5} (2.56^{-5}–1.13^{-4})	3.31^{-4} (1.17^{-4}–9.21^{-4})
IgG_2a	3.59^{-5} (1.66^{-5}–1.13^{-4})	1.58^{-4} (9.71^{-5}–3.07^{-4})
α_2-Macroglobulin	4.85^{-6} (1.81^{-6}–8.25^{-6})	2.89^{-5} (1.73^{-5}–5.06^{-5})

Table 3. Medians (ranges) of fractional plasma-to-urine clearances for β_2-microglobulin, orosomucoid, albumin, IgG_2a and α_2-macroglobulin in puromycin aminonucleoside (PAN) nephrosis rats before and after treatment with lysine

Protein	Before lysine treatment	After lysine treatment
β_2-Microglobulin	1.20^{-1} (8.89^{-2}–$2.14^{-1)}$	5.18^{-1} (2.75^{-1}–9.03^{-1})
Orosomucoid	4.18^{-2} (2.40^{-2}–7.56^{-2})	6.09^{-2} (3.69^{-2}–7.56^{-2})
Albumin	3.83^{-2} (2.03^{-2}–7.97^{-2})	5.91^{-2} (3.20^{-2}–3.21^{-1})
IgG_2a	2.26^{-2} (8.37^{-3}–6.98^{-2})	2.79^{-2} (1.61^{-2}–4.53^{-2})
α_2-Macroglobulin	1.51^{-3} (7.48^{-4}–1.55^{-3})	5.16^{-3} (4.25^{-3}–6.41^{-3})

used and the plates were incubated in a humid chamber at 8°C. The incubation time was three days for albumin and orosomucoid and ten days for IgG_2a. Purified albumin, IgG_2a and orosomucoid (Sigma) were used as standards.

The plasma and urine concentrations of β_2-microglobulin were determined by the radioimmunosorbent technique and the protein was purified according to Berggård and Bearn [25].

α_2-Macroglobulin (Scipac Limited, Sittingborn, UK) was fractionated by gel chromatography using a Superose 6 gel filtration column (Pharmacia Biotech, Sollentuna, Sweden). The fraction containing α_2-macroglobulin was bound to ^{125}I (Kjeller, Norway) by the Chloramine T method. The free ^{125}I was reduced from $\approx$2% to 0.2–0.5% using Amicon microfilters (Microcon 30). Urine samples containing radioactive tracer were precipitated using 10% trichloroacetic acid, and measurements were only performed on the precipitate that was rinsed twice to completely remove all free ^{125}I from the pellet. Radioactivity was measured using a gamma scintillator (Clini-Gamma - 1272; LKB-Wallac, Turku, Finland).

Calculations

Analyses of glomerular clearances as a function of molecular radius were performed according to the two-pore theory of membrane permeability and were described in detail previously [3, 26]. The formulas are presented in the **Appendix**.

In brief, clearances of all proteins larger than the assumed small-pore radius, as predicted from sieving curves of the smallest molecules investigated, can be shown to be determined solely by convective processes, and hence, their large-pore clearances are just products of the large-pore volume flow and the drag coefficients of the solutes [1–5, 8]. The small pore clearances can be determined from the non-linear (Patlak) flux equation [27].

Calculations and data fits were performed on a personal computer (Casio 880P) using an iterative procedure to get the best curve fit to the experimental data.

Statistical methods

Renal function, plasma and urinary data are given as medians and ranges. The Mann-Whitney *U*-test was used to compare sets of analyses. The level of significance was $P < 0.05$.

RESULTS

Glomerular filtration rate (GFR) and plasma levels of proteins in control rats and in nephrotic rats are shown in Table 1. The median plasma-to-urine clearances for the investigated proteins before and after lysine treatment in control rats are presented in Table 2 and in rats with PAN induced nephrosis in Table 3. With lysine treatment there was a statistically significant ($P < 0.05$) increase in the clearances of proteins in normal rats compared to the untreated animals. The clearance of large proteins increased five- to sixfold, with an exception for the clearance of orosomucoid, which was just doubled. The β_2-microglobulin clearance increased by a factor of 450. Puromycin aminonucleoside nephrosis (PAN) resulted in a marked (150 times) increase of the clearances of the large proteins. Lysine treatment of PAN nephrotic rats, however, resulted only in a slight and non-significant increase in clearances of these proteins.

In Figure 1 the measured plasma-to-urine clearances for the investigated proteins are plotted versus molecular radius in a semilogarithmic diagram and the data are analyzed using a two-pore curve fit. We were surprised to find a relatively sharp restriction of solute transfer as a function of increases in molecular radius for both small and large pores. The small-pore radius was determined to be 29 Å in all experiments. The large-pore radius was determined to be 115 Å, and ω_0 to $1.7 \cdot 10^{-4}$ in lysine-treated normal rats and 110 Å and $2.5 \cdot 10^{-2}$ in lysine-treated PAN nephrotic rats, respectively.

There is no direct indication for the presence of a shunt pathway from our data. However, we had no ability to analyze solutes larger than α_2-macroglobulin in radius because of the expected extremely low concentrations of such solutes. Thus, if the presence of a shunt pathway is postulated, it could maximally carry a flow of $2.89 \cdot 10^{-5}$ ml/min through the glomerular membrane, corresponding to the clearance of α_2-macroglobulin. Therefore, if a shunt pathway of this type is invoked, the large-pore estimate is reduced from 115 to 90 Å. Again, it should be pointed out that such a shunt pathway cannot be directly predicted from the present data.

In the PAN-induced nephrotic syndrome, where tubular reabsorption was inhibited and where ω_0 increased by a factor of 150, the small-pore radius (29 Å) and large-pore radius (115 Å) were

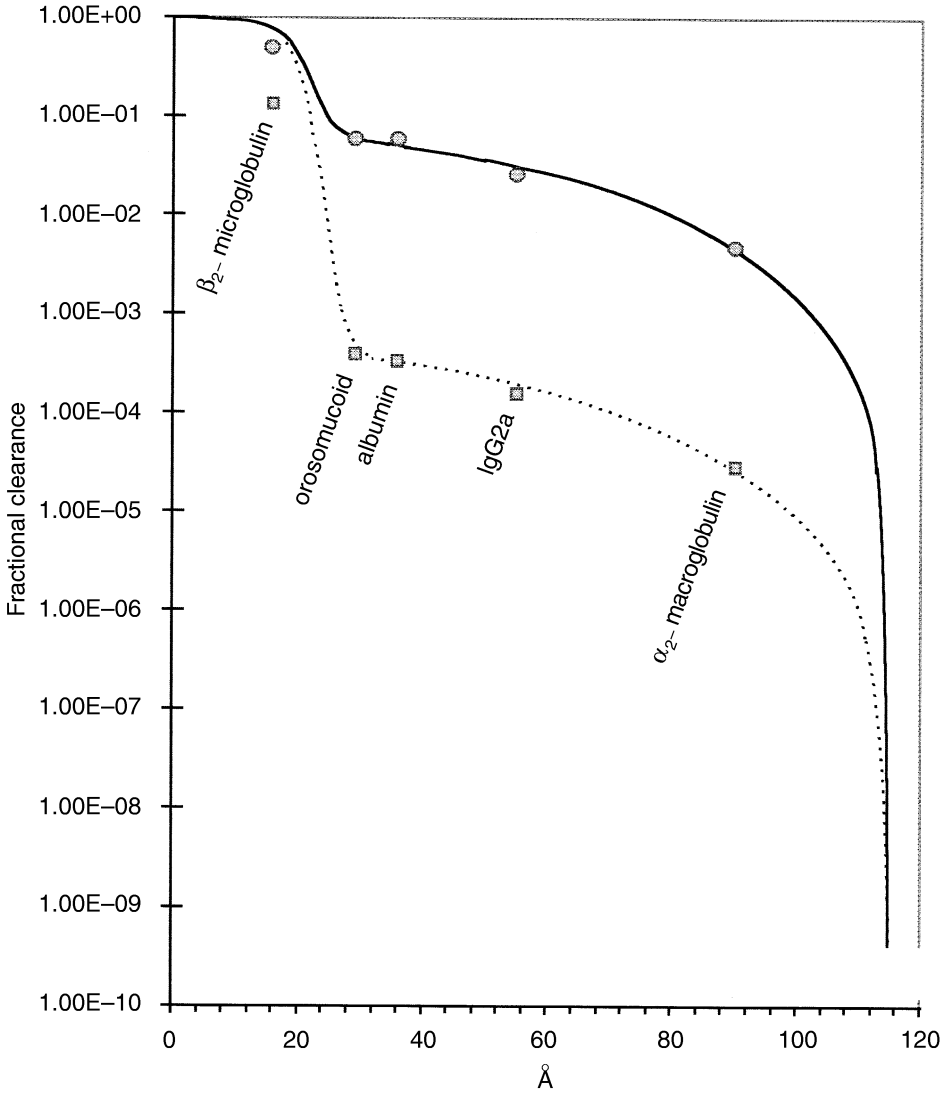

Fig. 1. Measured fractional plasma to urine clearances of β_2-microglobulin, orosomucoid, albumin, IgG$_2$a, and α_2-macroglobulin versus molecular radius in a semilogarithmic diagram. The data are analyzed using a two-pore curve fit. Lines are: (solid) PAN + lysine; (dotted) control + lysine.

unchanged compared to controls. Indeed, in the nephrotic syndrome the number of large-pores increased 170-fold while the small-pore system was more or less unaffected. The ratio between the number of large-pores and small-pores is according to Porseuille's law:

$$\frac{n_L}{n_S} = \frac{\omega_0}{1 - \omega_0}\left(\frac{r_S}{r_L}\right)^4$$

This ratio was calculated to be $\approx 6.9 \cdot 10^{-7}$ normally and, thus increased to $1.03 \cdot 10^{-4}$ in the nephrotic syndrome.

The tissue studies of the kidneys of the lysine-treated rats compared to untreated animals showed that the cytoplasm of the tubular epithelium was vacuolated and contained large amounts of silver-positive protein resorption droplets. In contrast, the appearance of the glomeruli was not different from that in normal animals.

DISCUSSION

The two-pore model of capillary permeability has been successfully applied to describe the blood-tissue transfer of small and large solutes in a large number of different organs [1–3]. Characteristically the large-pore radius has been found to be of the order of 250 Å in most continuous and fenestrated vascular beds, as judged from lymphatic flux analyses. To make such analyses one has to assess the filtrate-to-plasma concentration ratios of very large solutes, such as α_2-macroglobulin and IgM. However, with regard to glomerular permselectivity, most analyses do not include measurements of such large macromolecules. Most fractional clearances (or ultrafiltrate-to-plasma concentration ratios) have been analyzed for solutes up to the size of 60 to 70 Å [11]. Thus, they have often been assigned an infinite size, but larger than 80 Å [28]. Only very seldom have larger molecules been assessed. Still, glomerular permselectivity is nowadays usually analyzed

using a heteroporous concept, the large pores usually denoted as 'shunt-pathways' [12]. Strictly speaking, however, 'shunt pathways' denote pathways through which the fractional clearance of solutes above a certain size should be constant, but recent studies indicate that this may not be true [13, 29].

We performed the present study to get more insight into the size of the large-pore population in the glomerular barrier by analyzing urine protein fluxes after inhibition of proximal tubular protein reabsorption using lysine. Our data indicate that there are normally no non-discriminatory shunt pathways in the glomerular barrier, at least not of functional importance. If still existing, they may at maximum contribute 10^{-5} of the total glomerular hydraulic conductance (K_f).

The present data are not directly comparable to data obtained by glomerular sieving of neutral dextrans. Neutral dextrans are not spherically shaped, but are characterized by an elongated shape with a random-coil configuration. Endogenous macromolecules should be more ideal test probes for evaluating glomerular selectivity. Also, Ficoll, which is spherical in shape, has been recently used to compute membrane pore size parameters. These new measurements of size selectivity characteristically yield a small-pore radius of around 30 Å [11]. The main purpose of this study was to determine the radius of the large pores rather than the small pores' radius. However, according to our calculations, the effective, equivalent, small pore radius can at maximum be 29 Å, corresponding to a maximal uncharged radius of 44 to 45 Å. According to the Debye-Hückel theory of charged solute interactions, a 29 Å (radius) molecule will behave as a 37 Å (= 29 + 8) uncharged molecule, and a 45 Å (radius) pore will behave as a 37 Å (= 45 − 8) uncharged pore [30]. Since albumin has a molecular radius of 36 Å, it should normally not be able to filter through the small pores, or at least a majority of small pores, but should share more or less the same pathway as IgG and other large macromolecules. However, this pathway cannot be a shunt pathway, because in that case, large IgG losses to the urine should always be accompanied by significant urinary losses of even larger proteins, such as α_2-macroglobulin and IgM. This is almost never seen clinically. In the nephrotic syndrome, however, the fractional excretion of albumin and IgG is usually increased in parallel [31] (unpublished data), indicating an increased number of size-discriminative large-pore pathways shared by both albumin and IgG.

One implication of the presence of size-discriminative large pores in the glomerular filter is that, among other things, IgM will not pass the glomerular filter under normal conditions at all. Indeed, nearly all significant amounts of α_2-macroglobulin and IgM that are excreted to the urine should be derived from postrenal sources [32]. The present data cannot, however, rule out the presence of a very small number of shunt pathways accounting for at maximum $2 \cdot 10^{-5}$ of the total filtration rate. In that case the dominant large-pore pathway, besides the 29 Å small-pores, is a 90 Å large-pore from which α_2-macroglobulin is totally excluded. If the large-pore size is of the order of 90 to 115 Å, then an electrical charge will indeed affect transport across these pores, unlike the situation in 250 Å pores or shunt pathways. Both charge-selectivity and size-selectivity are thought to affect the transport of large molecules across the glomerular membrane. However, in the present PAN induced nephrosis, θ for albumin and θ for IgG increased in parallel, suggesting that charge alterations did not occur in the present experiments.

Lysine inhibition of proximal tubular reabsorption of proteins is usually considered to be more or less complete [10]. In the present experiments most macromolecule studied increased their clearance to the final urine by a factor five to six, while the change for β_2-microglobulin was 450-fold. Still, we expected further inhibition. The morphology studies that we performed on kidneys of lysine treated rats showed no glomerular pathology.

Another way of reducing the impact of proximal tubular reabsorption on protein transfer to the final urine is to saturate the reabsorption mechanism via the induction of massive proteinuria. Thus, ten rats were treated by PAN and investigated in exactly the same way as the control lysine-treated rats. We were surprised to find almost exactly the same protein excretion pattern as a function of molecular radius in both the lysine treated groups, except that large pore transport was greatly enhanced in the PAN nephrosis rats. This indicates that even though the lysine inhibition may not have been absolutely complete, the excretion pattern (and large-pore estimate) was similar in the two situations when tubular reabsorption was inhibited in different ways. Furthermore, the sieving coefficient for albumin obtained after lysine infusion in control rats was not very different from that obtained in isolated perfused kidneys where the tissue temperature had been reduced to 8°C, which should cause complete inhibition of proximal tubular reabsorption [4, 5]. We therefore feel quite confident that the pattern of clearance versus molecular radius found in the present study really mimics the normal sieving pattern in the Bowman's capsule ultrafiltrate. At most, the real ultrafiltrate concentration of proteins may be approximately 30 to 40% higher in absolute terms than measured in the present study. However, there is no reason to believe that the relative sieving pattern should be markedly different than that measured in the current study.

The present data may also be simulated using a log-normal (plus shunt) model. Such a model would, however, not give such a distinct sieving pattern for solutes ranging in size between 20 and 30 Å. However, applying this model the average equivalent pore size will be reduced from ≈30 Å to ≈20 to 25 Å. Since we lack the computer program to do these simulations, we have chosen not to apply the distributed model in the present pore estimations.

In summary, we have found a clear-cut size-restriction of molecules larger than albumin across the glomerular barrier, indicating the presence of 'large pores' in the glomerular membrane and not infinite 'shunt pathways.' Since the large pores have a smaller radius than previously thought, charge selectivity should be of importance not only in the small pores but also for macromolecular transport across the large pores [33, 34]. Moreover, if classic shunt pathways still exist, they contribute less than 10^{-5} of the total GFR. The major implications of our findings are that very large macromolecules like IgM will not pass the glomerular filter under normal conditions at all, whereas α_2-macroglobulin will appear only in extremely low concentrations. If IgM (or α_2-macroglobulin) appear in the appreciable amounts in the urine they can be expected to have been derived from postrenal sources rather than from glomerular filtration.

APPENDIX

Clearances of all proteins larger than the assumed small-pore radius, as predicted from sieving curves of the smallest molecules investigated, can be shown to be determined solely by convective

processes (see below), and hence, their large-pore clearances (Cl_L) are just products of the large-pore volume flow (Jv_L) and the drag coefficients (1-σ) of the solutes [1–5, 8]:

$$Cl_L = Jv_L(1 - \sigma)_L \qquad \text{(Eq. 2a)}$$

where σ denotes the reflection coefficient of the membrane to the particular solute. The sieving coefficient (θ) is then:

$$\theta = \frac{Jv_L}{GFR}(1 - \sigma)_L \qquad \text{(Eq. 2b)}$$

Here $(1 - \sigma)_L$ is determined in terms of solute radius (a_e) over the large pore radius (r_L), denoted λ, as can be described by current hydrodynamic theories for σ. Thus, according to the study of Mason, Wendt and Bresler [35] we have:

$$1 - \sigma = \frac{(1 - \lambda)^2[2 - (1 - \lambda)^2](1 - \lambda/3)}{1 - \lambda/3 + 2/3\lambda^2} \qquad \text{(Eq. 3)}$$

Hence, if the clearances of at least two solutes larger than the small-pore radius are measured it is possible from equations 2a and 3 (or Eq. 2b and 3) to determine both Jv_L (or θ) and r_L. Small-pore volume flow (Jv_S) is obtained by just subtracting Jv_L from GFR.

Knowing Jv_L and the hydraulic conductance of the membrane, it is possible to calculate the fractional hydraulic conductance accounted for by the large pores (ω_0) using the following equation [3, 26]:

$$Jv_L = \omega_0(1 - \omega_0)K_f(1 - \sigma_L)\Delta\pi + \omega_0 GFR \qquad \text{(Eq. 4)}$$

Here K_f is the hydraulic conductance of the glomerular membrane, σ_L is the protein reflection coefficient for albumin through large pores, and $\Delta\pi$ is the transvascular osmotic pressure gradient, which is equal to ≈26 mm Hg for a plasma colloid osmotic pressure of 22 to 23 mm Hg in rats (that is, lower than in humans). At a GFR of 1.4 ml/min, as in this study, K_f can be calculated to be approximately 0.14 ml/min/mm Hg, assuming a net transglomerular pressure gradient of 10 mm Hg in our rats. One then arrives at exactly the same K_f value per nephron (30,000 nephrons/rat) as obtained by Bohrer et al [36]. Inserting this value into 4, for example (which is a quadratic expression), for a Jv_L of 0.00065 ml/min yields $\omega_0 = 1.7 \times 10^{-4}$.

We now turn to the small pore clearances (Cl_S). These clearances can be determined from the non-linear (Patlak)-flux equation [27]

$$Cl_S = Jv_S(1 - \sigma)\frac{1 - (C_u/C_{pw})e^{-Pe}}{1 - e^{-Pe}} \qquad \text{(Eq. 5)}$$

where Pe, which represents a modified Peclet number, is defined as:

$$Pe = \frac{Jv_S(1 - \sigma_S)}{PS_S}$$

and PS_S (the solute permeability-surface area product in small pores) is defined as:

$$PS_S = \frac{D_S}{\Delta X}A = \frac{D_S}{\Delta X}\frac{A}{A_0}A_0 = D_S\frac{A_0}{\Delta X}\frac{A}{A_0} \qquad \text{(Eq. 6)}$$

where A represents the effective pore area available for restricted diffusion and A_0 is the total unrestricted cross-sectional pore area, and $\frac{A}{A_0}$ is a term describing the diffusion restriction of a solute in a (cylindrical) pore. $\frac{A_0}{\Delta X}$ represents the unrestricted pore area over unit diffusion distance. The free solute diffusion coefficient D_s is defined by:

$$D_S = \frac{RT}{6\pi \cdot N \cdot a_e \cdot \eta} \qquad \text{(Eq. 7)}$$

Here RT is the product of the universal gas constant and the temperature in degrees Kelvin, and finally, N is Avogadro's number, while η is water viscosity (0.007^{-1} dynes $\cdot$ $s^{-1} \cdot cm^{-2}$). $\frac{A}{A_0}$ is determined by the solute radius over small-pore radius $\left(\frac{a_e}{r_s}\right)$. We employed the expression [35]:

$$\frac{A}{A_0} = \frac{(1 - \lambda)^{9/2}}{1 - 0.3956\lambda + 1.0616\lambda^2} \qquad \text{(Eq. 8)}$$

to model the diffusion restriction as a function of solute radius over pore radius. $\frac{A_0}{\Delta X}$ can, according to pore theory, be determined using the following equation:

$$\frac{A_0}{\Delta X} = \frac{8\eta K_f}{r^2 \cdot 60 \cdot 1{,}320} \qquad \text{(Eq. 9)}$$

Here, 1,320 is a factor converting millimeters of mercury (mm Hg) into dynes per square centimeter, while 60 converts minutes to seconds. K_f represents the hydraulic conductance (the product of hydraulic conductivity and membrane surface area).

Thus, for r_S = 29 (Å) (see below) and K_f = 0.14 ml/min/mm Hg we can determine $\frac{A_0}{\Delta X}$ to $1.177 \cdot 10^6$ cm. Alternatively, $\frac{A_0}{\Delta X}$ and r_S can be determined as the best fit to experimental data of a specific (small pore) clearance versus mol radius curve.

According to Porseuille's law, the fractional large pore to small pore surface area ratio $\left(\frac{A_L}{A_S}\right)$ is determined by ω_0, r_S and r_L according to:

$$\frac{A_L}{A_S} = \frac{\omega_0}{1 - \omega_0}\left(\frac{r_S}{r_L}\right)^2 \qquad \text{(Eq. 10)}$$

Hence, for r_S= 29 (Å), r_L = 110 (Å) and ω_0 = 0.00017 and A_L/A_S becomes $1.18 \cdot 10^{-5}$ and $\frac{A_0}{\Delta X}$ for the large pore system = 13.82 (cm). It is now possible to test the validity of equations 2a and 2b versus equation 5, as applied for the large pore system. It is then evident that transport through large pores is convective to its nature. Thus, for albumin the Peclet number becomes 3.23 and for IgG_2a 10.7, that is, both above 3, indicating convection dominated transport.

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