Mechanisms of Proteinuria in Diabetic Nephropathy II

A Study of the Size-Selective Glomerular Filtration Barrier

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SUMMARY

We evaluated the size-selective properties of the glomerular barrier in 30 patients in whom diabetic nephropathy was associated with urinary IgG losses. Neutral dextrans of graded size were used to characterize glomerular membrane-pore structure. A fractional IgG clearance (relative to freely permeable inulin) smaller or greater than 0.001 was used to distinguish patients with minor (group 1, N = 14) and major (group 2, N = 16) urinary IgG leakage, respectively. Fractional clearances of dextrans (θ_D) of smaller size (radii 20–40 Å) were similar, but those of larger dextrans (radii 42–60 Å) were elevated in group 2 relative to group 1 patients. When plotted on log-normal probability coordinates, the correlation between θ_D and radius in healthy subjects is linear, suggesting that glomerular pores form one population with a normal distribution. In diabetic nephropathy with urinary IgG leakage, however, θ_D for large molecules was elevated and departed from linearity, suggesting a bimodal pore size distribution within the glomerular membrane. A pore model of solute transport revealed (1) the upper pore mode was highly permeable to large dextrans equivalent in size to IgG and (2) the fraction of glomerular filtrate permeating the large pores was greater in group 2 than in group 1 patients with diabetic nephropathy, 6% versus 3%, respectively. We conclude that urinary IgG leakage in diabetic nephropathy is determined by the development of a subpopulation of enlarged pores. The magnitude of urinary IgG losses appears to be a function of the membrane area-fraction occupied by the enlarged pores. DIABETES *32* (Suppl. 2):40–46, 1983.

The glomerular capillary wall, while permitting a high rate of filtration of water and small solutes, possesses a remarkable ability to restrict permeation of plasma proteins the size of albumin and larger into Bowman's space.[1,2] Ultrastructural tracer studies indicate that passage of proteins and other macromolecules through the three-layered glomerular capillary wall takes place through an extracellular route.[3,4] This extracellular pathway consists of the endothelial fenestrae, the glomerular basement membrane, the interpedicular filtration slit diaphragms and the filtration slits.[5] The role of a filtration barrier has been proposed for each succeeding structure along this pathway.[5] However, ultrastructural and cytochemical studies have revealed that the endothelial fenestrae and filtration slits are lined by a sialoglycoprotein coat that virtually occludes the latter space.[6] This coat and the adjacent glycoprotein-rich basement membrane may be regarded as a continuous anionic polymeric matrix extending from the base of the endothelial fenestrae to Bowman's space. This unit is capable of excluding macromolecules on the basis of their size and charge.[5]

Changes in both the structure and composition of the glomerular capillary wall have been detected within a few years of the onset of type I (insulin-dependent) diabetes. These have included progressive thickening of the glomerular basement membrane[7-9] and reduction of sialoglycoprotein content.[10,11] Despite these early physicochemical changes, the filtration properties of the diabetic glomerular capillary wall remain relatively normal for many years. A normal or high rate of glomerular ultrafiltration is maintained, permselectivity toward neutral dextran macromolecules is not different from that of the normal glomerulus, and leakage of large plasma proteins into the urine is negligible.[12,13] However, after many years substantial proteinuria eventuates and striking changes in the intrinsic membrane properties of the glomerular capillary wall become demonstrable. These include (1) profound depression of glomerular filtration rate (GFR) due primarily to severe reduction of the glomerular ultrafiltration coefficient[14] and (2) the development within the glomerular membrane of a small number of large pores or defects that are highly permeable to plasma proteins and neutral polymers of large size.[13]

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TABLE 1
Glomerular function in diabetic patients with urinary IgG losses

	Fractional clearance		Urinary excretion		
	IgG	Albumin	IgG	Albumin	Inulin clearance
	← × 10^{-5} →		← μg/min →		(ml/min/1.73 m^2)
Group 1 (N = 14)					
Mean	50	250	235	4331	53
SEM	8	61	42	865	7
Group 2 (N = 16)					
Mean	941	1887	917	6191	17
SEM	187	362	191	1364	6
P value 1 versus 2		<0.001	<0.005	NS	<0.001

Our understanding of the mechanisms governing the permselective properties of the glomerular capillary wall derives, in part, from fractional clearances of macromolecules, in which the clearance of a test macromolecule is compared with that of a freely permeable reference marker, such as inulin. If both the macromolecule and the reference marker are neither secreted nor reabsorbed, then the fractional clearance of the macromolecule is equivalent to the ratio of the concentration of macromolecule in the glomerular filtrate to its concentration in plasma water. That ratio describes its sieving coefficient. Polydisperse neutral dextran meets the foregoing criteria for an ideal test macromolecule. It can be used, therefore, to characterize the sieving properties of the glomerulus. The following study defines sieving properties of the glomeruli of type I diabetic patients with demonstrable urinary losses of the large plasma protein, immunoglobulin G (IgG).

METHODS

Patient population. Diabetic nephropathy was diagnosed in 30 patients because of insulin-requiring diabetes mellitus of more than 10 yr duration, heavy proteinuria (>1 g/24 h), and ophthalmoscopic evidence of diabetic retinopathy. All had easily measurable levels (>20 μg/ml) of IgG in unconcentrated urine. They were arbitrarily divided into two groups by the magnitude of urinary IgG leakage. We assumed that in the presence of heavy proteinuria the fraction of filtered IgG reabsorbed in the proximal tubule was minor or negligible.[15,16] Based on this assumption the clearance of IgG relative to freely permeable inulin (θ_{IgG}) was used as a measure of glomerular permeability to IgG. Accordingly, 14 patients in whom θ_{IgG} was below 0.001 were regarded as having minor urinary IgG leakage and designated as group 1. In the remaining 16 patients, $\theta_{IgG} > 0.001$. They were regarded as having major urinary IgG leakage and comprised group 2.

Study protocol. Each patient was studied by a differential macromolecule clearance technique described in detail elsewhere.[13,14] The study protocol was approved by the Committee for the Protection of Human Subjects in Research at Stanford University. Each patient gave informed consent before study.

Clearances were performed during water or furosemide diuresis. A priming dose followed by a constant infusion of inulin was administered. Dextran-40 (130 mg/kg) was infused intravenously immediately after the inulin prime. After a 60-min equilibration period, three carefully timed urine collections were taken. Glomerular filtration rate (GFR) was expressed as the mean inulin clearance of all three timed collections.

Fractional clearances (θ) of macromolecule probes (relative to inulin clearance) were determined during the first timed collection. The macromolecules used were albumin, IgG, and dextran-40. Albumin and IgG have effective molecular radii (r) of 36 and 55 Å, respectively. Dextran-40 is polydispersed over an r-range of 20–60 Å. Albumin and IgG concentrations were determined by radial immunodiffusion.[17] Dextran and inulin (the reference molecule) were assayed by an autoanalyzer anthrone technique. To permit θ for narrow dextran fractions to be determined (r interval = 2 Å), a protein-free filtrate of plasma and a urine sample were subjected to gel permeation chromatography before assay. The chromatographic technique and assay procedures employed in this study have been described previously.[13,14,17]

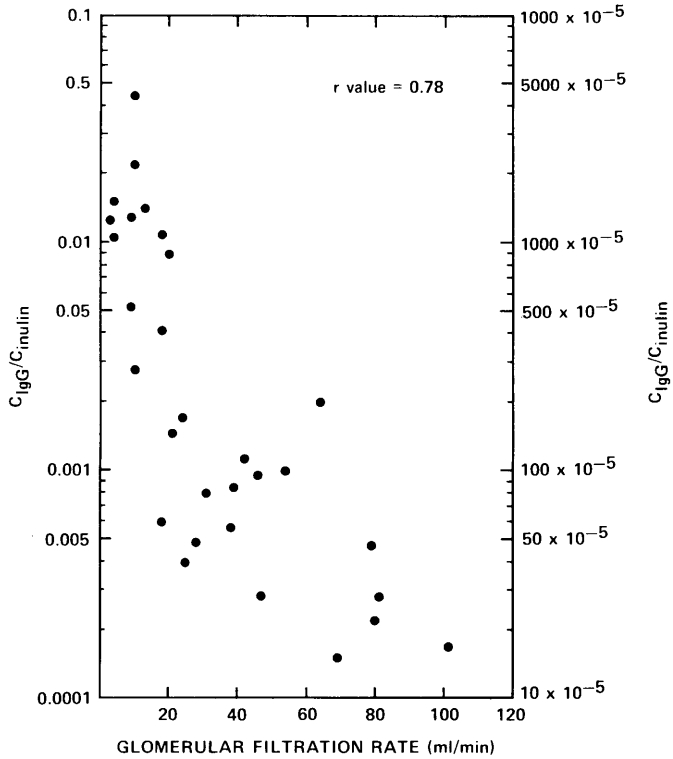

FIGURE 1. Relationship between fractional IgG clearance (θ_{IgG}) and glomerular filtration rate in proteinuric patients with diabetic nephropathy.

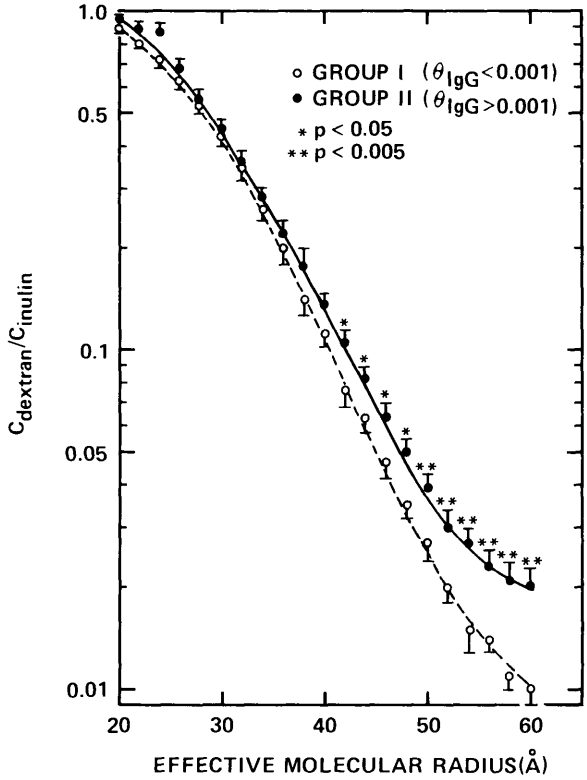

FIGURE 2. Fractional dextran clearance profile (or dextran sieving curve) for the glomerulus in diabetic subjects with minor (○) and major (●) urinary IgG leakage. All results are expressed as mean ± SEM. * = P < 0.05; ** = P < 0.005.

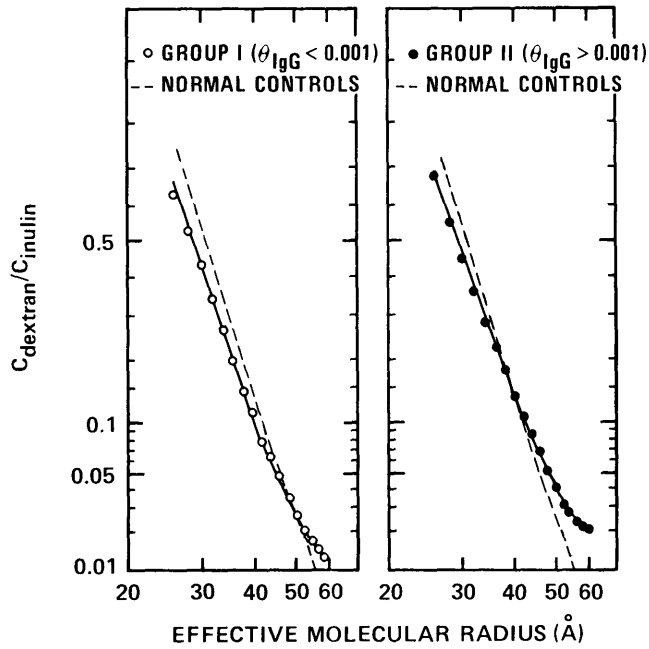

FIGURE 3. Fractional dextran clearances (or dextran sieving coefficients) plotted as a function of effective dextran radius on log-normal probability coordinates for diabetic subjects with minor urinary IgG leakage (○, left) and major urinary IgG leakage (●, right). The mean dextran sieving curve of 15 healthy volunteers is shown for comparison in each panel (interrupted line). Note that each diabetic sieving curve intersects the normal curve and that the latter is linear on these coordinates, whereas the diabetic sieving curves are curvilinear.

Statistical analysis. All results are expressed as the mean ± standard error of the mean. Student's *t* test for unpaired data was used to evaluate the significance of intergroup differences observed.

RESULTS

Glomerular filtration of water and proteins (Table 1, Figure 1). With one exception, GFR was reduced in each of the diabetic patients with detectable urinary IgG losses. Moreover, GFR reduction in individual patients was correlated with the magnitude of θ_{IgG} (Figure 1, correlation coefficient = 0.78). On average, GFR was threefold more depressed in patients with major (group 2) than with minor (group 1) urinary IgG leakage, 17 ± 6 versus 53 ± 7 ml/min/1.73 m^2, respectively (Table 1, $P < 0.001$). Fractional albumin clearance (θ_{alb}) paralleled θ_{IgG} and was considerably enhanced in those with major relative to minor urinary IgG leakage, 0.019 ± 0.003 versus 0.003 ± 0.001, respectively ($P < 0.001$).

Permselective properties of the glomerulus (Figures 2–5). Fractional clearance (θ_D) for a given dextran can be equated with the sieving coefficient of the glomerulus for that dextran. These values in 14 patients with minor ($\theta_{IgG} < 0.001$) urinary IgG leakage are compared with those of the remaining 16 patients with major urinary IgG leakage ($\theta_{IgG} > 0.001$) in Figure 2. The sieving coefficients for dextran molecules of relatively small r (20–40 Å) were similar in the two groups. However, sieving coefficients for larger dextran molecules (r = 42–60 Å) were significantly higher with major than with minor urinary IgG leakage, and this difference became magnified with increasing dextran size (Figure 2).

The changes in dextran and plasma protein clearance that accompany diabetic nephropathy with urinary IgG leakage can be explained by assuming a bimodal pore-size distribution within the glomerular membrane, with the major fraction of the glomerular filtrate passing through the small-pore component (assumed to be essentially IgG impermeable) while the large-pore component, very limited in area, is solely responsible for IgG leakage. This "two-population" membrane model is amenable to mathematical analysis in which the experimentally determined sieving curves are manipulated to allow independent determination of the solute- and solvent-transport characteristics of the two membrane populations.

According to this model (described in the APPENDIX), variation of the experimentally measured sieving coefficients with r can, to a reasonable approximation, be regarded as the arithmetic sum of solute transport through the small- and large-pore component of the membrane respectively, expressed as

$$\theta' \simeq \theta_\alpha + \theta_\beta\phi \qquad (1)$$

where θ' is the overall sieving coefficient for the whole glomerular membrane; θ_α and θ_β are the sieving coefficients for the small-pore and large-pore portions of the membrane, respectively; and ϕ represents the fraction of the total GFR that permeates the large-pore membrane area. It is evident from equation 1 that if the functional relationship between θ_α and r could be independently established, it would be possible to isolate the properties of the large-pore part of the

glomerular membrane by difference, and thereby estimate its area fraction, ϕ, and characteristic sieving curve, θ_β.

Such a manipulation of the sieving data is rendered feasible by applying the observation that sieving curves for the normal glomerular capillary wall appear as straight lines on log-normal probability coordinates.[13,18,19] An example of this relative linearity between θ_D and r on log-normal probability coordinates is shown in Figure 3. The interrupted line represents the mean dextran sieving curve of 15 healthy volunteers. In contrast, when dextran sieving coefficients are plotted as a function of r in either group 1 or group 2 patients with diabetic nephropathy (left and right panels), the sieving curve departs from linearity on these coordinates. This is true irrespective of the magnitude of urinary IgG leakage. Thus, in both group 1 and group 2 patients diabetic nephropathy was associated with an upward displacement of the large radius portion of the dextran sieving curve with the result that the relationship between θ_D and r was curvilinear (Figure 3).

Since at small values of r the sieving coefficient for the glomerulus in diabetic nephropathy is dominated by the contribution of the small-pore part of the membrane (i.e., $\theta' \simeq \theta_\alpha$), on log-normal probability coordinates, the low-r and linear part of the sieving curve is taken to be equivalent to θ_α. This construction is shown for group 2 diabetic nephropathy in the left panel of Figure 4. In this case, the difference between the actual curve θ' and the linear approximation, θ_α, is very nearly equal to $\theta_\beta\phi$ (equation 1).

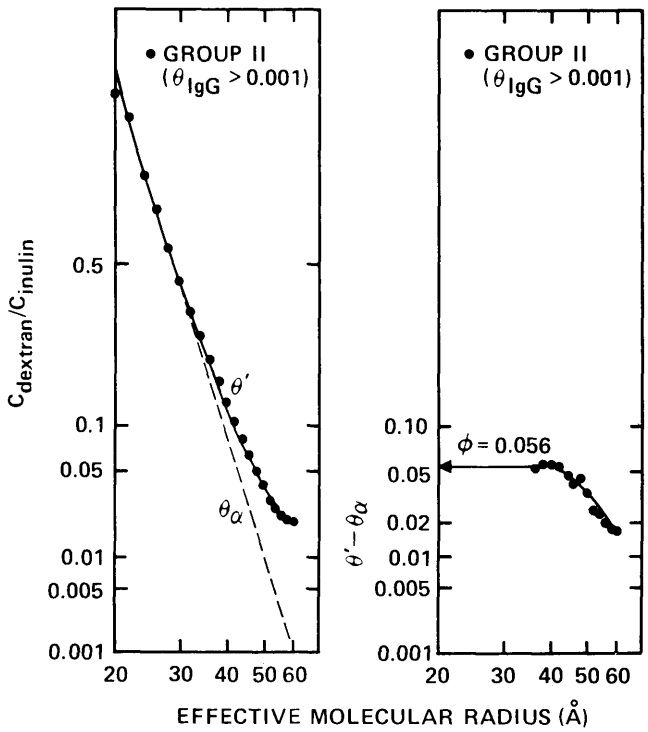

FIGURE 4. Left panel: The dextran sieving curve for diabetic subjects with major urinary IgG leakage is plotted on log-normal probability coordinates. Right panel: The difference between θ' and θ_α is plotted as a function of effective dextran radius. The asymptotic value, 0.056, is equal to ϕ, the fraction of total GFR permeating the large pores (for detailed explanation see text). θ' = Overall sieving curve for the whole glomerular membrane (uninterrupted line); θ_α = sieving curve for the small-pore component of the glomerular membrane (interrupted line).

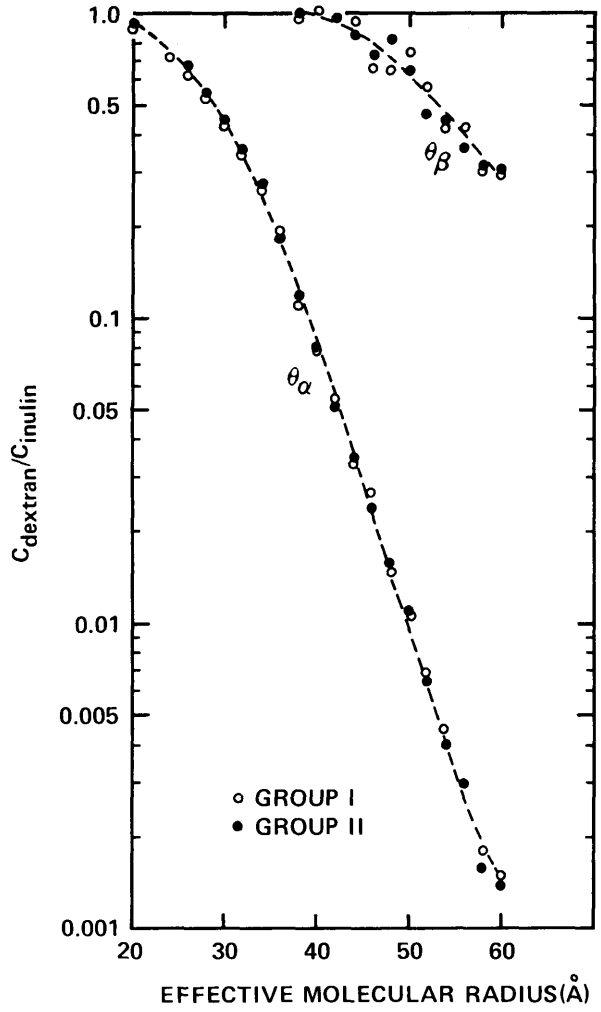

FIGURE 5. Computed values for the sieving curves of the small-pore (θ_α) and large-pore (θ_β) regions of the glomerular membrane in proteinuric diabetic nephropathy. Sieving coefficients in those with minor or major urinary IgG leakage are depicted by open and solid circles, respectively.

It now remains to independently determine ϕ and the sieving curve for the IgG-permeable part of the membrane, θ_β. This can be done by first plotting ($\theta' - \theta_\alpha$) versus r on log-normal coordinates (right panel, Figure 4). Since, as $r \rightarrow 0$, θ_β must approach 1.0, this curve must approach a limiting asymptote equal to ϕ. In this case, ϕ, the fraction of total GFR permeating the larger pores, approximates 0.056. When plotted in the manner of Figure 4, $\phi \simeq 0.033$ in group 1 patients. Thus, minor (group 1) and major (group 2) urinary IgG leakage seem to be distinguished by a twofold difference in the fraction of total GFR that permeates the larger pores, 3% versus 6%, respectively.

Once ϕ is determined, dividing the computed values of $\theta_\beta\phi$ by ϕ obviously yields the sieving curve for the large-pore component of the glomerular membrane, θ_β. The values for θ_α and θ_β predicted by the foregoing computations are illustrated in Figure 5. Dextran transport through the smaller pores was restricted for molecules with r larger than 20 Å (i.e., $\theta_\alpha < 1.0$). In the larger pores by contrast, restricted dextran transport ($\theta_\beta < 1.0$) only became evident once dextran r exceeded 40 Å. Judged by a comparison of θ_β and θ_α for

the largest dextran molecules examined, the larger pores were two orders of magnitude more permeable than the smaller pores. Note, however, that the computed values of θ_α and θ_β were nearly indistinguishable between the patient groups with minor and major urinary IgG leakage, respectively. It may be that pore dimensions are similar in the two populations, but that as suggested by the larger value for ϕ, a greater membrane area-fraction is occupied by larger pores in group 2 patients, and this accounts for the enhanced dextran and IgG filtration observed.

DISCUSSION

Sustained proteinuria is the clinical hallmark of diabetic nephropathy. There is, however, a lag of many years between the onset of type I diabetes and the development of sustained proteinuria.[20–22] In the decade or more that typically precedes the appearance of overt diabetic nephropathy small but transient increases in urinary excretion of radioimmunoassayable albumin have been detected. This phenomenon has been referred to as microalbuminuria.[23,24] As evidenced by normal sieving of neutral dextrans, microalbuminuria cannot be attributed to an alteration in the size-selective properties of the glomerular capillary wall.[12,13] Either a loss of charge selectivity or altered glomerular hemodynamics may be implicated in the genesis of this trivial and reversible form of early proteinuria.

Depletion of glomerular sialoglycoproteins during the course of diabetic nephropathy is predicted to result in reduction of the density of fixed negative charges in the glomerular capillary wall.[10,11] Accordingly, the transglomerular passage of circulating polyanions, including the relatively small protein albumin (r = 36 Å, isoelectric point at pH = 4.7), is likely to be enhanced. Defective electrostatic barrier function is unlikely, however, to account for the increased transglomerular passage of IgG, the major subclasses of which are neutral or only weakly charged (isoelectric point = 6.6–8.0).[25] Moreover, determinations of the effective molecular dimensions of IgG[26,27] and of mean pore size of the glomerulus,[18,28,29] respectively, reveal both to have a radius of approximately 55 Å, with the result that IgG should be effectively excluded from Bowman's space on the basis of its size alone. The presence of large quantities of IgG in the urine of patients with diabetic nephropathy therefore suggests an alteration in the size-selective properties of the glomerular capillary wall.[18]

In an effort to characterize glomerular sieving of diabetics with heavy urinary IgG losses, we studied the transglomerular passage of neutral dextrans. Notwithstanding greatly enhanced leakage of large plasma proteins into the urine, transport of neutral dextrans with effective molecular r between 24 and 40 Å was restricted relative to normal glomeruli (Figure 3). By contrast, the transport of larger dextran molecules (r = 50–60 Å) was enhanced, suggesting that the membrane in diabetic nephropathy has a less sharp "cut-off" than normal for large molecules (Figure 3).

When plotted on log-normal probability coordinates, the variation of experimentally measured dextran sieving coefficients with r in normal volunteers is described by a straight line (Figure 3). This finding is in accord with earlier observations in normal man[18,30] and in various mammalian species including the rat[19] and the rabbit.[31] The same also holds true for diabetic patients without proteinuria.[13] This type of sieving coefficient correlation implies a reasonably narrow unimodal distribution of pore size.[19] Any significant departure from linearity on these coordinates, as evidenced by diabetic patients with heavy proteinuria (Figure 3), may thus be construed to indicate a bimodal or more complicated distribution of pore size within the glomerular membrane.[19]

For the sake of simplicity, we have assumed a bimodal pore-size distribution and employed a model of the glomerular membrane in the advanced stage of diabetic nephropathy that provides a means for interpreting the observed changes in the transglomerular transport of polydisperse neutral dextrans in terms of a change in membrane-pore structure. According to this model, the pathologic glomerular membrane of diabetics with heavy proteinuria is composed of a parallel array of two radically different pore structure components. The main component, i.e., the larger part of the total glomerular membrane area, is a "small-pore" ultrafilter, similar in ultrastructure to that of the normal glomerular membrane, and is responsible for the retention of dextrans of small r. The minor component, i.e., the smaller part of the total membrane area, is postulated as being a "large-pore" ultrafilter, through which large (and small) macromolecules are able to penetrate. Judged by the fraction of total GFR that passes through the large-pore component (defined as ϕ) much less than 6% of the total surface area of the glomerular membrane is composed of such "defective membrane." Moreover, the value for ϕ is computed to be twice as large with major as with minor urinary IgG leakage (6% versus 3%, respectively), suggesting that the magnitude of transglomerular IgG filtration is a function of the membrane area-fraction occupied by large pores.

In attempting to relate the foregoing findings to proteinuria, it should be emphasized that the dextrans used to define membrane-pore structure differ from proteins not only with respect to charge but also in configuration. Clearance techniques in the rat suggest that plasma proteins are substantially more restricted by the normal glomerular capillary wall than dextrans of equivalent size and similar charge.[32] This disparity has been ascribed to asphericity and molecular compliance of dextran molecules under shear, with the result that their effective molecular dimensions during transglomerular permeation are smaller than those estimated by gel permeation chromatography.[33] If the clearance of proteins and equivalent-sized dextrans through the glomerular membrane in patients with diabetic nephropathy is similarly disparate, it remains unclear whether the greater enhancement of albumin than IgG passage (Table 1) takes place through the large-pore region of the membrane alone, or through a charge-depleted small-pore component of the membrane as well.

Less uncertainty surrounds the portal of IgG entry into Bowman's space and hence into urine. The computed sieving coefficients of the small-pore component of the membrane (θ_α) for dextrans similar in size to IgG approach 0.001 (Figure 5). If, because of its globular shape, IgG is considerably more restricted than equivalent-sized dextran, it seems likely that the small pores must be virtually impermeable to IgG. In contrast, the computed sieving curve for the large-pore component of the membrane reveals dextran in the r

interval 50–60 Å to be highly permeant (θ_β = 0.3–0.6, Figure 5), whence this region of the membrane is predicted to be quite permeable to IgG as well.

Although the urinary excretion and fractional clearance of albumin in diabetic nephropathy is larger than the corresponding values for IgG, the latter protein is, in our view, a more reliable probe of the size-selective filtration barrier of the glomerular capillary wall. Whereas modest and selective albuminuria has been observed without changes in membrane-pore structure, enhanced urinary IgG leakage in diabetics can invariably be related to the development of a subpopulation of enlarged pores in the glomerular membrane.[13] Furthermore, the magnitude of urinary IgG leakage, and hence the sum of all the large pores that have developed in the glomerular membrane during the course of its deterioration, is inversely related to GFR. This latter correlation may prove to be a useful predictor of irreversible glomerular damage. Further study is required to determine whether the considerable disruption of glomerular function and structure associated with urinary IgG leakage can be attenuated by meticulous maintenance of normotension and normoglycemia, or whether such glomerular dysfunction will progress irrevocably to end-stage renal failure.

APPENDIX

For a parallel-array of two structurally different membranes, water and solute flow through the glomerular wall can be described as follows:

Let $J_{w(\alpha)}$ = water flux through the retentive (small-pore) membrane area (ml · cm^{-2} · s^{-1}) and $J_{w(\beta)}$ = water flux through the IgG permeable (large-pore) area. Then

$$GFR = J_{w(\alpha)} A_{(\alpha)} + J_{w(\beta)} A_{(\beta)} \quad (A1)$$

where $A_{(\alpha)}$ and $A_{(\beta)}$ are the areas of small- and large-pore components of the membrane, respectively, (in cm^2) and

$$A_{(\alpha)} + A_{(\beta)} = A_{(T)} \quad (A2)$$

where $A_{(T)}$ is the total glomerular membrane area in cm^2.

Let $\phi = \dfrac{J_{w(\beta)}A_{(\beta)}}{J_{w(\alpha)}A_{(\alpha)} + J_{w(\beta)}A_{(\beta)}} = \dfrac{J_{w(\beta)}A_{(\beta)}}{GFR}$ = constant, where ϕ = fraction of GFR that permeates the large pore membrane area. Hence

$$J_{w(\beta)}A_{(\beta)} = GFR \cdot \phi \quad (A3)$$

and

$$J_{w(\alpha)}A_{(\alpha)} = GFR\ (1 - \phi) \quad (A4)$$

Now, let J_S be the flux of a specified macrosolute through the glomerular membrane under conditions in which its plasma concentration is C_p. If $\theta_{(\alpha)}$ is the sieving coefficient of that solute by the retentive (small-pore) membrane area, and $\theta_{(\beta)}$ its value for the large-pore membrane area, mass conservation requires that

$$J_S = \theta' C_p\,(GFR) = \theta_{(\alpha)} C_p J_{w(\alpha)} A_{(\alpha)} + \theta_{(\beta)} C_p J_{w(\beta)} A_{(\beta)} = C_F(GFR) \quad (A5)$$

where C_F = solute concentration in the glomerular filtrate and θ' = overall sieving coefficient of that solute for the glomerular membrane ($\theta' = C_F/C_P$).

Combining equations A3, A4, and A5 we have

$$\theta' = \theta_{(\alpha)}(1 - \phi) + \theta_{(\beta)}\phi \quad (A6)$$

Now, both $\theta_{(\alpha)}$ and $\theta_{(\beta)}$ decline from unity toward zero as the size of permeating solute molecule increases, although $\theta_{(\alpha)}$ does so far more rapidly than $\theta_{(\beta)}$; under these circumstances, the following limiting simplifications of equation A6 apply:

$$\lim_{\theta_{(\beta)} \to 1.0} \theta' = \theta_{(\alpha)} + \phi[1 - \theta_{(\alpha)}] \quad (A7)$$

$$\lim_{\theta_{(\alpha)} \to 0} \theta' = \theta_{(\beta)}\phi \quad (A8)$$

If, in addition $\phi \simeq 0$, then equation A6 reduces to

$$\theta' \simeq \theta_{(\alpha)} + \theta_{(\beta)}\phi \quad (1)$$

where $\theta_{(\alpha)} = f_{(\alpha)}r$, and $\theta_{(\beta)} = f_{(\beta)}r$, where $f_{(\alpha)}$ and $f_{(\beta)}$ are the functional relationships between θ and r of the permeating molecule for the two types of membrane.

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