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Mechanisms of proteinuria in diabetic nephropathy: A study of glomerular barrier function

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Mechanisms of proteinuria in diabetic nephropathy: A study of glomerular barrier function. The fractional clearance of neutral dextrans (θ_D) with Einstein-Stokes radii between 30 and 64 Å was determined in normal subjects (controls, $N = 15$) and in diabetic patients with heavy proteinuria (advanced nephropathy, $N = 16$) or trace proteinuria (early nephropathy, $N = 8$). When plotted on log normal probability coordinates, the correlation between θ_D and radius in controls and in early diabetic nephropathy was linear, suggesting that glomerular pores form one population with a normal distribution. In advanced diabetic nephropathy, however, θ_D for large molecules (radius > 46 A) was elevated and departed from linearity suggesting a bimodal pore size distribution within the glomerular membrane. A mathematical model was devised, which revealed the mean fraction of glomerular filtrate permeating the upper pore mode to be 0.009 ± 0.002, and the pores to be totally nondiscriminatory toward molecules with radii up to 64 Å. We conclude that the development of large pores (or defects) within the glomerular membrane in advanced diabetic nephropathy permits the unrestricted passage of large plasma proteins into the urine.

Mécanismes de la protéinurie au cours de la néphropathie diabétique: étude du fonctionnement de la barrière glomérulaire. La clearance fractionnelle de dextrans neutres (θ_D) ayant des rayons d'Einstein-Stokes entre 30 et 64 Å était déterminé chez des sujets normaux (contrôles, $N = 15$) et chez des diabétiques ayant une protéinurie abondante (néphropathie évoluée, $N = 16$) ou à l'état de traces (néphropathie débutante, $N = 8$). En coordonnées log normales, la corrélation entre θ_D et le rayon était linéaire chez les normaux et au cours des néphropathies diabétiques débutantes suggérant que les pores glomérulaires constituent une population de distribution normale. Cependant, au cours de la néphropathie diabétique évoluée, θ_D pour les grosses molécules (rayon > 46 Å) était élevée et s'écartait de la linéarité, suggérant une distribution bimodale de la taille des pores de la membrane glomérulaire. En utilisant un modèle mathématique, on a constaté qu'en moyenne la fraction de la surface représentée par les pores de grande taille était de 0,009 ± 0,002, et que ces pores ne discriminaient en rien les molécules dont le rayon atteignait 64 Å. Nous concluons que l'apparition de larges pores (ou defects) dans la membrane glomérulaire au cours de la néphropathie diabétique évoluée laisse un libre passage aux grosses protéines plasmatiques dans l'urine.

The magnitude of proteinuria may be used as a measure of the severity of diabetic nephropathy. When a sensitive radioimmunoassay has been applied to urine samples of patients with insulin-dependent diabetes of recent onset, a slight but measurable increase in urinary albumin excretion (20 to 100 μg/min) was observed [1–3]. Ten or more years are likely to elapse after the development of diabetes, however, before urinary albumin excretion exceeds 100 μg/min, a value above which conventional tests of proteinuria become positive [4–6]. This increased proteinuria heralds a gradual deterioration in glomerular function [7]. An insidious but progressive decline in glomerular filtration rate (GFR) to levels resulting in endstage renal failure is accompanied by increasingly heavy proteinuria, which eventually reaches nephrotic proportions [7–10].

Typical of the most advanced stage of diabetic nephropathy is a nonselective proteinuria with massive leakage into the urine of large plasma proteins, the most copious of which are albumin and immunoglobulin G (IgG) [11, 12]. 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 [13, 14]. Accordingly, the transglomerular passage of circulating polyanions, including albumin (isoelectric point = 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) [15]. Moreover, determinations of the effective molecular dimensions of IgG [16, 17] and of mean pore size of the glomerulus [18–20] 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 urine samples of patients with diabetic nephropathy, therefore, is suggestive of an alteration in the size-selective properties of the glomerular capillary wall [20].

Our understanding of the mechanisms governing the permselective properties of the glomerular capillary wall derive, in part, from fractional clearances of macromolecules, in which the clearance of a test macromolecule is compared to 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 its glomerular filtrate-to-plasma water concentration ratio. That ratio may be represented by the sieving coefficient (θ). Polydisperse neutral dextran which meets the foregoing criteria for an ideal test macromolecule can be used, therefore, to characterize the sieving properties of the glomerulus [21].

In an earlier study of nephrotic patients with diabetic nephropathy we found that relative to normal subjects, the

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transglomerular passage of neutral dextrans with Einstein-Stokes radii (r) between 20 and 40 Å was restricted, whence it appeared that mean glomerular pore size might be paradoxically reduced. The glomerular sieving coefficient of dextrans with r = 40 to 48 Å, however, approached or slightly exceeded that in normal subjects [11]. This evidence that the glomerular membrane in diabetic nephropathy has a less sharp "cut-off" than normal for large molecules is consistent with a bimodal distribution of pore size, the upper mode being relatively permeable to large macromolecules with molecular dimensions similar to those of large plasma proteins [2]. To test this hypothesis, we studied transglomerular dextran transport in another group of patients with diabetic nephropathy. We also devised a mathematical model of the glomerular membrane to provide a means for *(1)* interpreting changes in transglomerular dextran transport in terms of membrane-pore structure and *(2)* relating such transport changes to proteinuria.

Methods

Rationale. As stated previously, the observed changes in dextran and plasma protein clearance which accompany the advanced stage of diabetic nephropathy can be rationalized by assuming the formation of a bimodal pore-size distribution within the glomerular membrane, with the major fraction of the glomerular filtrate passing through the small-pore component of the distribution (which is essentially IgG impermeable), while the minor large-pore component 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 dtermination of the solute- and solvent-transport characteristics of the two membrane populations. (See Appendix.)

A dextran-sieving curve representative of the glomerular membrane in advanced diabetic nephropathy [11] is shown in Figure 1. The *solid curve* describes the variation of the experimentally measured sieving coefficients with r. As discussed in the Appendix, this curve 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, and expressed as

$$\theta' \cong \theta_\sigma + \theta_\beta\phi \qquad \text{Eq. 1}$$

where θ' is the overall sieving coefficient for the whole glomerular membrane, θ_σ 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 which permeates the large-pore membrane area. θ_σ and $\theta_\beta\phi$ are depicted as *dashed lines* in the left panel of Figure 1. It is evident from this construction that, if the functional relationship between $\theta_{(\sigma)}$ 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, $\theta_{(\beta)}$.

Such a manipulation of the sieving data is rendered feasible by applying the observation that sieving curves for most natural and synthetic ultrafiltration membranes including the glomerular capillary wall, plot as straight lines on log-normal probability coordinates [20, 22]. Because 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 [that is, $\theta' \cong \theta_{(\sigma)}$], on log-normal probability coordinates the low-r part of the sieving curve should be linear and equivalent to $\theta_{(\sigma)}$. The construction of Figure 1 then appears as shown in the right hand panel. In this case, the difference between the actual curve θ' and the linear approximation, $\theta_{(\sigma)}$, is very nearly equal to $\theta_{(\beta)}\phi$ (Equation 1).

It now remains to independently determine ϕ and the sieving curve for the IgG permeable part of the membrane, $\theta_{(\beta)}$. This can be done by first plotting $[\theta' - \theta_{(\sigma)}]$ vs. r on log-normal coordinates. Since, as $r \to 0$, $\theta_{(\beta)}$ must approach 1.0, this curve must approach a limiting asymptote equal to ϕ (Fig. 1, right panel). Once ϕ is determined, dividing the computed values of $\theta_{(\beta)}\phi$ by ϕ obviously yields the desired sieving curve, $\theta_{(\beta)}$.

Patient population. Twenty-four patients with insulin-dependent diabetes mellitus of more than 10 years' duration and ophthalmoscopic evidence of diabetic retinopathy were studied by a differential macromolecule clearance technique. They were arbitrarily separated into two groups by the absence or presence, respectively, of heavy proteinuria (>1 g/24 hr). Eight patients comprised the group with slight or absent proteinuria and were designated early diabetic nephropathy. The remaining 16 patients had heavy proteinuria and were designated advanced diabetic nephropathy. Fifteen healthy volunteers, similar in age to patients with advanced diabetic nephropathy, and who were free of renal disease and normotensive, served as controls. The study protocol was approved by the Stanford University Committee for the Protection of Human Subjects in Research. Informed consent was obtained from each patient and volunteer prior to study.

Study protocol. Each patient and volunteer voided spontaneously after water diuresis had been established by oral water loading. A priming dose followed by a constant infusion of inulin and para-aminohippurate (PAH) was administered to permit determination of the rates of glomerular filtration (GFR) and of effective renal plasma flow (ERPF) from the respective urinary clearances of each marker as described previously [23]. Dextran-40 (Rheomacrodex, Pharmacia Fine Chemicals, AB, Uppsala, Sweden) 130 mg/kg was administered by slow (~10 min) intravenous injection immediately following the inulin /PAH prime. At the end of a 40- to 60-min equilibration period, the bladder was emptied by voiding, after which three carefully timed 20- to 30-min urine collections were made. From urine and plasma obtained during the first of these timed collections, fractional clearances (or sieving coefficient) of Dextran-40 (θ_D) were computed, using the equation

$$\theta_D = [(U/P)_D]/[(U/P)_{in}] \qquad \text{Eq. 2}$$

where $(U/P)_D$ and $(U/P)_{in}$ refer to the urine-to-midpoint plasma concentration ratios of dextran and inulin, respectively. The same urine and plasma samples were used for the determination of the urinary excretion rate and plasma concentration of albumin and IgG respectively. GFR and ERPF were expressed as the mean value of all three timed urine collections.

Laboratory methods. For the calculation of GFR, inulin concentration of urine and plasma was determined using the autoanalyzer method of Fjeldbo and Stamey [24]. This method uses the fructose-specific reagent resorcinol, which is uninfluenced by the presence of dextran. The autoanalyzer method of Harvey and Brothers was used for the determination of PAH [25].

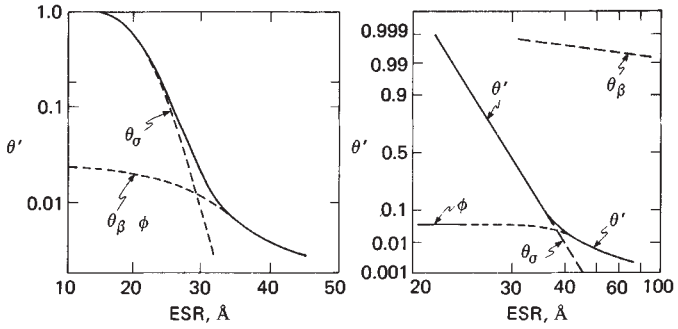

Fig. 1. *Hypothetical sieving curve for a glomerular membrane in advanced diabetic nephropathy plotted on semilogarithmic coordinates* (left panel) *and log-normal probability coordinates* (right panel). Symbols used are θ', overall sieving coefficient for the whole glomerular membrane; $(\theta)_\sigma$ and $(\theta)_\beta$ are sieving curves for small- and large-pore components of the glomerular membrane, respectively; ϕ, fraction of total GFR permeating large pores; ESR, Einstein-Stokes radius. For detailed explanation see text.

Separation of Dextran-40 and inulin in plasma and urine into narrow fractions (approximately 2 to 4 Å) was accomplished by gel permeation chromatography using a column (93.5 cm long × 1.6 cm I.D.) packed with Sephacryl S-300 and calibrated with three narrow dextran fractions of known molecular size provided by the manufacturer (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Using 0.3% buffered saline as eluent, 2.6 ml eluted fractions were collected with an automatic fractionator (Gilson Model S-80). The void volume (V_o) was determined with Blue Dextran, and the fractional volume available to the solute (K_{av}) was calculated as

$$K_{av} = (V_e - V_o)/(V_t - V_o) \qquad \text{Eq. 3}$$

where V_e is the elution volume of the solute and V_t the total volume of the gel column [26]. Einstein-Stokes radii (r) for individual dextran fractions were calculated from K_{av} [27]. Following gel permeation chromatography of plasma and urine, eluted fractions were assayed for dextran and inulin concentrations using a modification of the autoanalyzer anthrone method of Scott and Melvin [28]. Most of the eluted inulin had radii of 13 to 15 Å, and fractions containing molecules in this size range were used to calculate the $(U/P)_{in}$ ratio for subsequent determination of fractional clearances. It should be noted that $(U/P)_{in}$ as determined by the anthrone method was not significantly different from that obtained by the resorcinol method.

Urine samples were concentrated by between 25- and 100-fold and tested, respectively, against antisera specific for human IgG heavy chains and for lambda and kappa light chains (Meloy Labs Inc., Springfield, Virginia) using the double diffusion Ouchterlony technique [11]. Reactions of identity with all three antigens were the same as those obtained with an isolated preparation of human polyclonal IgG, indicating that heavy chain-reactive urinary IgG in patients with diabetic nephropathy represents the intact plasma protein. The concentrations of IgG and albumin in unconcentrated urine were then determined by radial immunodiffusion using low level immunodiffusion plates as described previously [11]. These plates permit the measurement of IgG and albumin over a concentration range of 1 to 25 and 4 to 100 mg/dl, respectively.

Statistical analysis. All results are expressed as the mean ± SEM. Student's t test for unpaired data was used to evaluate the significance of inter-group differences observed.

Results

Renal function and urine protein excretion (Table 1, Fig. 2). That the magnitude of proteinuria provides a reasonable basis for classifying patients with diabetic nephropathy into early and advanced categories is suggested by the respective values for GFR and ERPF. Whereas GFR and ERPF in patients with early diabetic nephropathy and slight proteinuria were similar to those in normal subjects (122 ± 20 and 412 ± 129 vs. 100 ± 9 and 469 ± 41 ml/min/1.73m^2, respectively); the corresponding values in patients with advanced diabetic nephropathy and heavy proteinuria were significantly depressed below normal to 27 ± 6 and 116 ± 24 ml/min/1.73m^2, respectively ($P < 0.01$). An equally striking relationship between magnitude of proteinuria and overall kidney function was observed in individual patients. Although the precise contribution of tubular protein reabsorption to the excretion rate of filtered proteins in patients with primary glomerular diseases is unknown, the rate at which a given protein is excreted per ml GFR may be regarded as a measure of glomerular permeability to that protein [29]. A plot of albumin and IgG excretion per ml GFR against GFR is illustrated in Figure 2. Each test protein was undetectable or present in trace quantities only in the urine of patients with early diabetic nephropathy and normal GFR. With advanced diabetic nephropathy, however, the observed rate at which each protein was excreted per ml GFR was proportional to the degree to which GFR was depressed. For all patients with advanced diabetic nephropathy and those with early diabetic nephropathy in whom protein excretion was measurable by radial immunodiffusion, the inverse relationship between the respective rates of albumin and IgG excreted per ml GFR and GFR was strongly correlated (r value = 0.90 and 0.78, respectively).

Fractional dextran clearances (Table 2, Figures 3 and 4). The sieving curve for dextrans in the r range 30 to 64 Å (K_{av} = 0.5 to 0.2) in advanced diabetic nephropathy was different from that in normal control subjects. The sieving coefficients for dextran molecules of relatively small r were depressed below normal. The opposite was true of sieving coefficients for dextrans of large r, which were elevated above normal (Fig. 3). These differences from normal were statistically significant for the smallest (r = 30 to 36 Å) and largest (r = 60 and 64 Å) dextrans examined (Table 2). In early diabetic nephropathy a slight but similar trend away from normality failed to reach statistical significance (Table 2). The small and large r ends of the sieving curve in advanced diabetic nephropathy were also respectively depressed and elevated relative to early diabetic nephropathy. Only the elevation of the sieving coefficients for the largest dextran fractions in advanced relative to early diabetic nephropathy, however, reached statistical significance (Table 2).

In addition to the absolute elevation of the large r portion of the sieving curve in patients with advanced diabetic nephropathy, its configuration was different from that in patients with early diabetic nephropathy or that of normal control subjects, respectively. When plotted on log-normal probability coordinates the relationship between θ_D and r approximated linearity

Table 1. Renal function and urinary protein excretion[a]

	Age	GFR	ERPF	$U_{Alb}V$	$U_{IgG}V$
	years	*ml/min/1.73m²*		*μg/min*	
Normal control (C)	49	100	469	2.7	0.5
(*N* = 15)	±5	±9	±41	±1.0	±0.3
Early DN	33	122	412	203	7.8
(*N* = 8)	±4	±20	±129	±110	±7.8
P vs. C	<0.02	NS	NS	NS	NS
Advanced DN	45	27	116	3,786	570
(*N* = 16)	±3	±6	±24	±475	±118
P vs. C	NS	<.01	<.01	<.001	<.001
P vs. early DN	<0.05	<.001	<.05	<.001	<.001

[a] All values are expressed as mean ± SEM.

Abbreviations are UV, urine excretion rate; NS, not significant; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; DN, diabetic nephropathy; $U_{Alb}V$, urinary excretion rates of albumin; $U_{IgG}V$, urinary excretion rates of IgG.

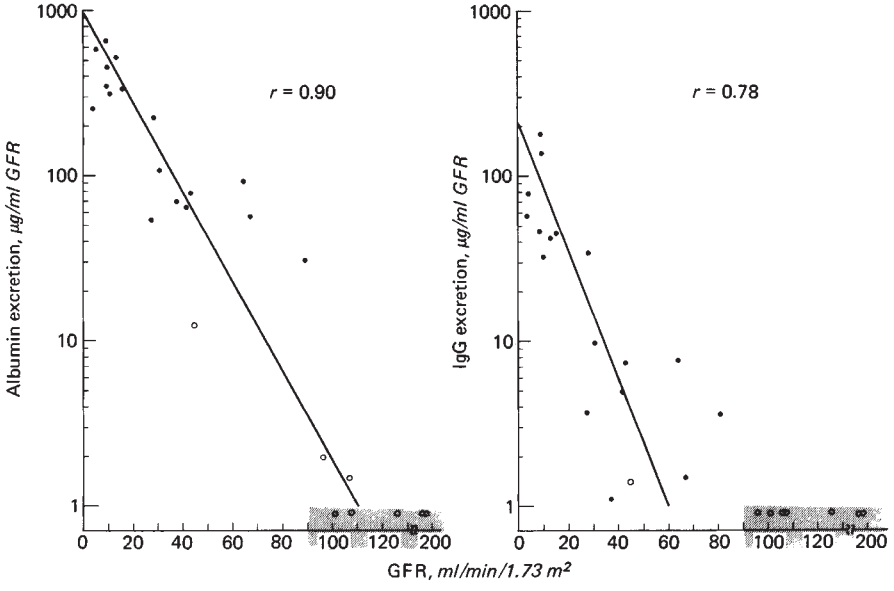

Fig. 2. *Relationship between protein excretion rate per milliliter glomerular filtrate and GFR in patients with diabetic nephropathy.* Albumin excretion is plotted in *left panel* and IgG excretion in *right panel,* respectively. Patients with advanced diabetic nephropathy are depicted as *closed circles* and those with early diabetic nephropathy as *open circles.* Unmeasurable protein excretion rates are depicted in the *shaded zone* in the lower right hand corner.

Table 2. Fractional dextran clearances

	30	32	34	36	38	40	42	44	46	50	53	57	60	64
						Å								
Normal controls (C)														
N = 15														
Mean	0.626	0.471	0.350	0.256	0.191	0.141	0.103	0.077	0.058	0.035	0.022	0.015	0.009	0.006
± SEM	0.040	0.031	0.023	0.016	0.012	0.009	0.007	0.005	0.004	0.003	0.002	0.003	0.001	0.001
Early DN														
N = 8														
Mean	0.475	0.378	0.297	0.237	0.181	0.139	0.105	0.081	0.061	0.039	0.025	0.016	0.011	0.007
± SEM	0.046	0.039	0.032	0.029	0.021	0.016	0.013	0.011	0.009	0.006	0.004	0.002	0.001	0.001
P vs. C	<0.25	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Advanced DN														
N = 16														
Mean	0.400	0.318	0.246	0.196	0.155	0.122	0.095	0.074	0.059	0.039	0.027	0.020	0.016	0.015
± SEM	0.029	0.024	0.020	0.015	0.013	0.011	0.009	0.007	0.006	0.004	0.003	0.003	0.002	0.002
P vs. C	<.001	<.001	<.005	<.02	NS	NS	NS	NS	NS	NS	NS	NS	<.005	<.001
P vs. early DN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<.05	<.005

Abbreviation is DN, diabetic nephropathy.

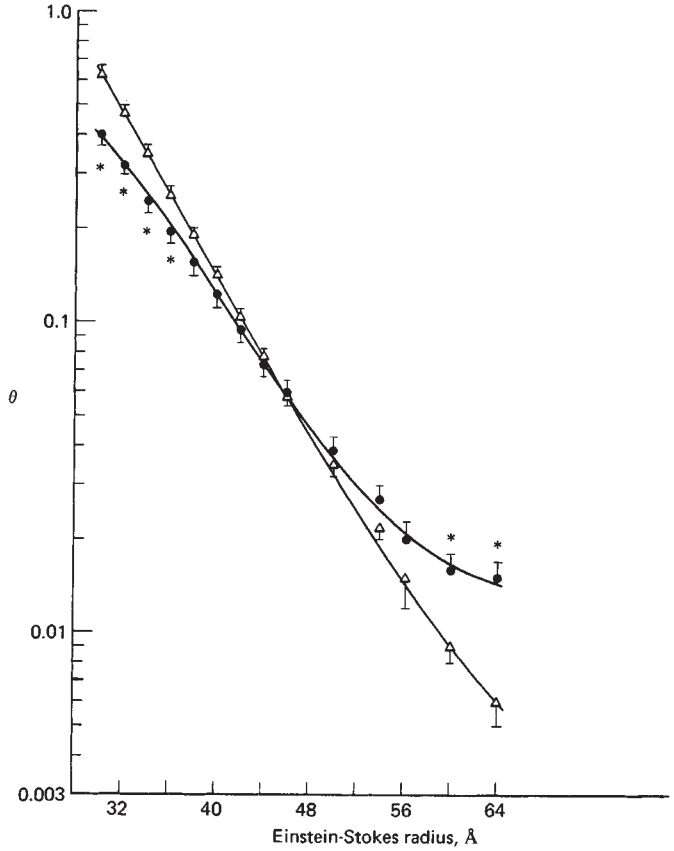

Fig. 3. *Fractional dextran profile (or dextran sieving curve) for the glomerulus in healthy volunteers* (△) *and in subjects with advanced diabetic nephropathy* (●). All results are expressed as mean ± SEM. The *asterisk* equals $P < 0.01$.

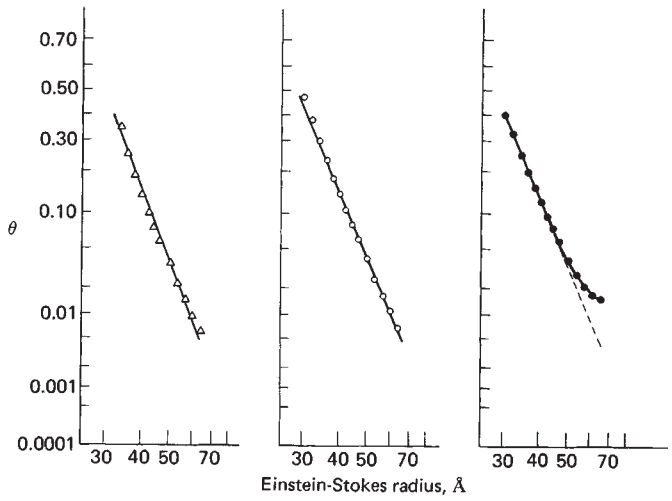

Fig. 4. *Fractional dextran clearances (or dextran sieving coefficients) plotted as a function of dextran Einstein-Stokes radius on log-normal probability coordinates for healthy volunteers* (△, left), *early diabetic nephropathy* (○, middle), *and advanced diabetic nephropathy* (●, right). The linear extrapolation *(or* θ_σ*)* in advanced diabetic nephropathy reflects the sieving properties of the small-pore component of the membrane.

in the latter two populations (Fig. 4). In advanced diabetic nephropathy, however, elevation of θ_D for molecules with $r >$ 46 Å resulted in an upward displacement of the latter part of the sieving curve with the result that the relationship between θ_D and r was curvi-linear (Fig. 4).

Analysis of glomerular macromolecule transport in advanced diabetic nephropathy (Table 3). The measured variation of θ_D with r (or θ'), and the linear extrapolation of the early part of the sieving curve (θ_σ) for each subject with advanced diabetic nephropathy was plotted on log-normal probability coordinates in the manner of Figure 1**B**. Upward deflection above linearity of three or more of the largest dextran fractions permitted the difference between θ' and $\theta_{(\sigma)}$ (or $\theta_\beta\phi$) to be estimated in 13 instances (Equation 1). From the limiting asymptote of the relationship between r and $\theta_\beta\phi$ the fraction of glomerular filtrate entering Bowman's space through an hypothetical upper mode of large pores, ϕ, was estimated to average 0.009 ± 0.002 (range 0.003 to 0.055). Dividing $\theta_\beta\phi$ by ϕ revealed the upper mode to be totally non-discriminatory with respect to dextrans of all sizes examined (that is, $\theta_\beta \approx 1.0$).

By assuming that like dextrans of equivalent size, albumin and IgG were also unrestricted, the predicted load of each protein filtered through an upper mode of large pores [$FL_{(\beta)}$] was estimated from the product of the respective plasma protein concentration and (Φ·GFR). This computation yielded values for $FL_{(\beta)}$ albumin of 5813 ± 1372 and for $FL_{(\beta)IgG}$ of 1780 ± 559 μg/min. These values are in excess of the measured urinary excretion rates of albumin (3813 ± 569) and IgG (542 ± 140 μg/min) by 1.5 and 3.3-fold, respectively.

Fractional reabsorption of filtered albumin and IgG have been shown to be similar [30]. The relatively small disparity between albumin excretion rate and its filtration rate through large pores may be an indication, therefore, of a larger filtered load of albumin than can be accounted for by permeation through an upper mode of large pores alone. In support of this possibility is the relationship between the individual values for $FL_{(\beta)}$ and protein excretion rate (Table 3). The measured rate of IgG excretion was similar to or less than $FL_{(\beta)IgG}$ in all 13 patients. By contrast, albumin excretion rate exceeded $FL_{(\beta)albumin}$ by 20 to 154% in five instances. Thus, whereas filtration through a small upper mode of nondiscriminatory pores could, in theory, account entirely for the observed rate of IgG excretion, an additional contribution to albumin filtration through the lower pore mode must be invoked to explain the observed rate of albumin excretion in some patients with advanced nephropathy.

Discussion

Proteinuria is widely regarded as the hallmark of diabetic nephropathy and is the commonest clinical manifestation in patients with diabetic glomerulosclerosis [31]. Its prevalence, however, does not parallel morphological alterations in the glomerular capillaries. Demonstrable widening of the glomerular basement membrane and expansion of the mesangium are evident by electronmicroscopy years before proteinuria becomes manifest [32, 33]; an even more striking alteration of these glomerular structures, easily diagnosed by light microscopy, has been reported in patients without proteinuria [31, 34–37]. From the long duration of insulin-dependent diabetes (>10 years) and the association with diabetic retinopathy, it seems probable that the eight patients categorized as early diabetic nephropathy in this study have structural abnormalities of their

Table 3. Analysis of glomerular macromolecule transport in advanced diabetic nephropathy

Patient no.	ϕ[a]	$FL_{(\beta)albumin}$[b]	$U_{albumin}$ V[c]	$FL_{(\beta)IgG}$[b]	U_{IgG} V[c]
		$\mu g/min$			
1	0.007	8,206	2,669	1,381	208
2	0.004	4,201	1,488	1,106	102
3	0.004	3,608	3,335	1,335	297
4	0.005	5,009	3,864	1,032	320
5	0.003	3,729	2,599	662	41
6	0.006	15,437	3,790	7,718	100
7	0.018	2,049	4,437[d]	1,303	1,341
8	0.006	1,885	3,075[d]	410	409
9	0.015	1,226	909	751	203
10	0.014	3,036	7,720[d]	1,436	1,600
11	0.007	5,292	6,351[d]	1,215	950
12	0.020	4,978	6,840[d]	603	537
13	0.055	16,915	2,487	4,193	942
$N = 13$					
Mean	0.009	5,813	3,813	1,780	542
± SEM	±0.002	±1,372	±569	±559	±140

[a] ϕ is the fraction of total GFR filtered through large pores.
[b] $FL_{(\beta)}$ is the predicted load of protein filtered through large pores, μg/min.
[c] UV is the urine excretion rate, μg/min.
[d] UV exceeds $FL_{(\beta)}$ for albumin.

glomerular capillaries [38–40]. However, as judged by a normal rate of water ultrafiltration, unremarkable permselective properties toward neutral dextrans and absent or scant protein leakage into the urine, no overt impairment of glomerular function could be detected. The relationship between glomerular structure and function in the early stages of diabetic nephropathy therefore remains obscure.

In contrast is the strong association between heavy proteinuria in advanced diabetic nephropathy and light microscopic changes typical of the diffuse and sometimes of the nodular form of diabetic glomerulosclerosis [31, 37]. The presumptive presence of severe diabetic glomerulosclerosis in the 16 patients categorized as advanced diabetic nephropathy provides a likely explanation for the observed reduction in the rate of water ultrafiltration. Thus a greatly expanded mesangial matrix, by obliterating many capillary lumina, would likely result in a reduction of surface area available for filtration [31]. As judged by enhanced filtration of large dextran molecules (>46 Å), there also exists a defect in the size-selective glomerular barrier to macromolecule filtration.

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* (Fig. 4). This finding is in accord with earlier observations in normal man [20, 41] and in various mammalian species including the rat [22] and the rabbit [42]. The same also holds true for patients with early diabetic nephropathy (Fig. 4). This type of sieving coefficient correlation implies a reasonably narrow unimodal distribution of pore size [22]. Any significant departure from linearity on these coordinates, as is evidenced by patients with advanced diabetic nephropathy, may thus be construed to indicate a bimodal or more complicated distribution of pore size within the glomerular membrane [22].

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 which 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 advanced diabetic nephropathy is composed of a parallel array of two radically different pore structure components. The main component (that is, 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 (that is, 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. The fraction of the total GFR, which passes through the "large-pore" component (defined as ϕ) is computed to be less than 0.01, meaning that much less than 1% of the total surface area of the glomerular membrane is composed of such "defective" membrane. The sieving curve computed for this damaged segment of the membrane ($\theta_{(\beta)}$ vs. r) suggests that dextran molecules of $r \leq 64$ Å are unrestricted in passage; whence, this membrane component is presumed to be highly permeable to plasma proteins as well.

Although the theoretical analysis from which these membrane parameters have been derived should not be regarded as a representation of glomerular morphology, it is interesting to note that at least two processes that might cause a focal disruption of the glomerular filtration barrier have been observed in advanced diabetic nephropathy. These are a focal foot process degeneration with detachment of epithelial cells from and denudation of the underlying glomerular basement membrane [43], and the development of electron lucent "moth-eaten" areas within the glomerular basement membrane [44]. Either process may provide a structural basis for large membrane defects which do not discriminate among size or charge of macromolecules and behave as an upper mode of large protein-permeable pores.

Our theoretical analysis requires two assumptions: *(1)* IgG (r = 55 Å) is too large to permeate the small-pore component of the membrane, and *(2)* the large-pore component does not discriminate between IgG and a dextran molecule of equivalent size. Based on these assumptions, the predicted load of IgG filtered through the large-pore component of the membrane ($FL_{(\beta)IgG}$) is of sufficient magnitude to account for the observed urinary excretion of IgG, which it exceeds by a factor of 3.3 on average. The fractional reabsorption of only two thirds of filtered IgG, implicit in the foregoing computation, is consistent with a saturable mechanism for the uptake of filtered proteins by the proximal tubule epithelium [45, 46]. It should be emphasized, however, that indirect 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 [47]. 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 [48]. If the clearance of proteins and equivalent-sized dextrans through the large-pore component of the glomerular membrane in advanced diabetic nephropathy is similarly disparate, then our computation of the load of IgG filtered through

large pores will be in excess of the true value, while the actual fraction of IgG reabsorbed will be correspondingly smaller.

There is now a large body of evidence which indicates that the glomerular capillary wall excludes macromolecules on the basis of charge as well as size [49–51]. Given the absence of a detectable upper mode of protein-permeable pores and tendency toward a shift in the distribution of pores to those of smaller size or lower density in early diabetic nephropathy, it is tempting to postulate that the relatively minor leakage of albumin into the urine is due to electrostatic barrier dysfunction. Thus, reduced electrostatic retardation of the relatively small (r = 36 A) but highly anionic albumin molecule will enhance its permeation across a glomerular capillary wall depleted of its fixed negative charges. The finding in some patients with advanced diabetic nephropathy that the rate of albumin excretion exceeds the predicted load of albumin filtered through the large-pore component of the glomerular membrane ($FL_{(\beta)albumin}$, Table 3) suggests that defective electrostatic barrier function in the small-pore component of the glomerular membrane also contributes to albuminuria in these latter patients. In keeping with this interpretation is the finding from cytochemical and compositional studies of glomeruli from patients with diabetic nephropathy that glomerular polyanion is depleted [13, 14].

We offer the following hypothesis, based on the foregoing analysis and observations, to explain the pathogenesis of proteinuria in diabetic nephropathy. During the early stages of the disease, progressive widening of the glomerular basement membrane may be accompanied by loss of fixed negative charges in the glomerular capillary wall. The relatively small initial rate of albumin leakage into the urine could result from an ensuing loss of electrostatic retardation. In keeping with the observations of others, it may be predicted from the Y intercept of the regression line for albumin excretion vs. GFR (Fig. 2) that albuminuria will develop while GFR is still within the normal range (112 ml/min) [1–3, 7]. Although urinary IgG excretion is not measurably enhanced at relatively high values of GFR, a modest increase in glomerular permeability with enhanced tubular reabsorption of this large protein cannot be excluded. With increasing glomerular damage, a second population of large pores develops within the glomerular membrane, and permits the unrestricted transmembrane passage of large plasma proteins, including IgG. From the Y intercept of the regression line for urinary IgG excretion vs. GFR, this upper mode of pores and hence IgG leakage into the urine is predicted to become apparent once GFR has declined to approximately 50% of normal (60 ml/min, Fig. 2). Hereafter progressive damage to both pore modes eventuates. As the small-pore mode becomes progressively depleted of its negative charges, the transglomerular transport of albumin and other anionic proteins of relatively small r becomes enhanced. Simultaneously, an increasing size and charge-independent flux of plasma proteins through the upper mode ensues, the rate of which is determined, in part, by the sum of all the defects (or large pores) that have developed in the membrane during the course of its deterioration. The appearance of massive proteinuria and the nephrotic syndrome as a near-terminal event during the evolution of diabetic nephropathy [8–10] implies that in the final stages of the lesion, the increase in protein clearance must be disproportionately large relative to the simultaneous reduction in GFR. It remains to be determined whether this terminal enhancement of fractional protein clearance is a consequence of *(1)* an absolute increase in the filtered protein load, or of *(2)* a decline in fractional reabsorption due to saturation of the tubular protein transport mechanism. It is tempting to speculate, however, that a combined contribution by each of these determinants of fractional protein clearance must be required to explain nephrotic-range proteinuria in diabetic nephropathy even when GFR has declined to values so low that the initiation of dialysis therapy must be contemplated.

Appendix

For a parallel-array of two structurally different membranes, water and solute-flow through the glomerular wall can be described as follows: Let $J_{w(\sigma)}$ = Water flux through the retentive (small-pore) membrane area (ml · cm^{-2} · sec^{-1}) and $J_{w(\beta)}$ = Water flux through the IgG permeable (large-pore) area. Then:

$$GFR = J_{w(\sigma)}A_{(\sigma)} + J_{w(\beta)}A_{(\beta)} \qquad \text{Eq. A1}$$

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

$$A_{(\sigma)} + A_{(\beta)} = A_{(T)} \qquad \text{Eq. A2}$$

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

$$\text{Let } \phi = \frac{J_{w(\beta)}A_{(\beta)}}{J_{w(\sigma)}A_{(\sigma)} + J_{w(\beta)}A_{(\beta)}} = \frac{J_{w(\beta)}A_{(\beta)}}{GFR} = \text{const}$$

Where ϕ = Fraction of GFR which permeates the large-pore membrane area.
Hence:

$$J_{w(\beta)}A_{(\beta)} = GFR \cdot \phi \qquad \text{Eq. A3}$$

and

$$J_{w(\sigma)}A_{(\sigma)} = GFR\,(1 - \phi) \qquad \text{Eq. A4}$$

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 $\theta_{(\sigma)}$ 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

$$J_S = \theta' C_P\,(GFR) = \theta_{(\sigma)}C_P J_{w(\sigma)}A_{(\sigma)} + \theta_{(\beta)}C_P J_{w(\beta)}A_{(\beta)} = C_F\,(GFR) \qquad \text{Eq. A5}$$

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:

$$\theta' = \theta_{(\sigma)}(1 - \phi) + \theta_{(\beta)}\phi \qquad \text{Eq. A6}$$

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

$$\lim_{\theta_{(\beta)} \to 1.0} \theta' = \theta_{(\sigma)} + \phi[1 - \theta_{(\sigma)}] \qquad \text{Eq. A7}$$

$$\lim_{\theta_{(\sigma)} \to 0} \theta' = \theta_{(\beta)}\phi \qquad \text{Eq. A8}$$

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

$$\theta' \simeq \theta_{(\sigma)} + \theta_{(\beta)}\phi \qquad \text{Eq. 1}$$

where $\theta_{(\sigma)} = f_{(\sigma)}r$, and $\theta_{(\beta)} = f_{(\beta)}r$, where $f_{(\sigma)}$ 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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