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Glomerular size-selectivity and microalbuminuria in early diabetic glomerular disease

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Glomerular size-selectivity and microalbuminuria in early diabetic glomerular disease. Sieving coefficients of uncharged dextrans of graded size (radii 30 to 60 Å) were used to characterize barrier size-selectivity in nonazotemic diabetic humans with microalbuminuria (Group 1, N = 11) or macroalbuminuria (Group 2, N = 21). Compared to a non-diabetic control group (N = 21) the low radius end of the sieving profile was depressed, whereas the high radius end was elevated in each diabetic group, more so in Group 2 than Group 1. A heteroporous membrane model revealed the major portion of the glomerular barrier to be perforated by restrictive pores of ~56 Å radius in all three groups. However, in keeping with a parallel trend for GFR, the relative density of restrictive pores was control > Group 1 > Group 2. The remaining minor portion of the barrier was perforated by large, shunt-like pores, the relative prominence of which ranked Group 2 > Group 1 > control. Although the hypothetical, fractional clearance of macromolecules attributable to the shunt-like pores varied directly with fractional clearances of albumin and IgG, the progressive increment in the latter fractional protein clearances in the two diabetic groups was disproportionate. This raises the possibility that factors in addition to barrier size defects contribute to the development, magnitude and composition of proteinuria early in the course of diabetic glomerular disease. Such factors could include: (1) a progressive decrement in fractional reabsorption as the filtered protein load increases, and (2) a concomitant loss of barrier charge-selectivity, permitting albumin but not IgG to escape through restrictive pores, and thereby accounting for an observed excess of albumin over IgG clearance in both micro- and macroalbuminuric stages of diabetic glomerular disease.

The sclerosing glomerular injury that complicates diabetes mellitus becomes clinically manifest by the development of proteinuria. By the time proteinuria is detectable by dipstick, the urinary excretion rate of albumin is usually in excess of 300 μg/min, a value 10 times higher than the upper limit of normal [1]. We and others have shown that proteinuria of this magnitude is accompanied by abnormal sieving behavior of glomeruli towards uncharged dextrans of broad size distribution [2–6]. The abnormality is characterized by enhanced filtration of large nearly impermeant dextran macromolecules, whereas filtration of dextrans of smaller molecular radius (r_s) is restricted. The selective enhancement of transglomerular passage of large dextrans has been inferred from pore theory to represent the formation in the glomerular capillary wall of a subset of enlarged pores [7]. Whereas such enlarged pores are computed to be few in number, they are estimated to be non-discriminatory towards dextrans of r_s up to 60 Å, and are likely, therefore, to be permeable also to albumin (r_s = 36 Å), thereby providing a possible basis for the phenomenon of albuminuria.

With increasingly widespread use of sensitive immunochemical assays over the past decade, it has become apparent that subtle elevation of the urinary albumin excretion rate into a pathological but dipstick negative range (30 to 300 μg/min) antedates the development of dipstick-positive proteinuria by several years. This phenomenon, known as microalbuminuria, is thought to represent the earliest detectable evidence of functional injury to the filtration barrier in diabetic glomerular disease (DGD).

It has been suggested that microalbuminuria might reflect a loss of barrier charge-selectivity as anionic sites in the diabetic glomerular capillary wall become depleted [8]. However, microalbuminuria has recently been shown to be accompanied by a parallel increase in the urinary excretion rate of immunoglobulin G (IgG), the second most copious of the plasma proteins [8]. The large size (r_s = 55 Å) and predominantly neutral or cationic molecular charge of IgG points to impairment of barrier size-selectivity, and makes it unlikely that the phenomenon of microalbuminuria can be explained entirely by an isolated failure of the glomerular capillary wall to function as a negatively-charged electrostatic barrier [7]. We have accordingly used uncharged dextrans to probe the intrinsic, size-selective properties of glomeruli in a group of patients with insulin-dependent diabetes mellitus (IDDM) and microalbuminuria. To determine whether evolution from an early microalbuminuric to a subsequent macroalbuminuric stage of DGD is associated with a graded impairment of barrier size-selectivity, we simultaneously studied a second group of overtly proteinuric, but non-azotemic patients with IDDM.

Methods

Patient population

We recruited for study patients with IDDM, in whom either dipstick positive proteinuria or a urinary excretion rate of immunoassayable albumin in excess of 30 μg/min was detected for the first time within the preceding 12 months. To ensure that the recent onset of enhanced albuminuria represented an early functional expression of diabetic glomerular disease, we included only patients in whom the prevailing level of serum creatinine was within the normal range (<1.4 mg/dl in males, <1.2 mg/dl in females). The presence of ophthalmoscopic evidence of diabetic retinopathy was used to make a clinical diagnosis of DGD. In three instances the diagnosis of diabetic

retinopathy was equivocal, and the presence of DGD was confirmed by histopathological demonstration of diffuse diabetic glomerulosclerosis in renal tissue obtained by a closed needle biopsy.

During a five year period (1986 through 1990) 32 patients with IDDM met the aforementioned functional and diagnostic criteria for early DGD. They were divided into two groups according to the level of albuminuria at the time of study. Group 1 consisted of 11 patients with microalbuminuria, which we have arbitrarily defined as between 30 and 300 μg/min. The remaining 21 patients had macroalbuminuria (>300 μg/min), and they comprise Group 2. The duration of IDDM in Groups 1 and 2 was 23 ± 2 and 20 ± 2 years (mean ± SEM) respectively, and the corresponding daily insulin requirement at the time of study averaged 44 ± 3 and 44 ± 5 U/day.

Twenty-one healthy volunteers were examined during the same five year period with identical physiological techniques and provided a control range for the glomerular functional qualities of interest. They spanned an age range (18 to 54 vs. 21 to 66 yr) and exhibited a sex distribution (10 males, 11 females) similar to that encountered in the patient population (15 males, 17 females). They had no history of renal disease, hypertension, or diabetes. At the time of evaluation each was found to be normotensive, normoglycemic and to have a negative dipstick for urinary protein.

Physiological determinations

Each patient and volunteer was studied in a clinical research center after giving informed consent to a protocol that had been approved previously by the Institutional Review Board at Stanford University. Differential solute clearances were performed during water diuresis 21 days after discontinuation of converting enzyme inhibitor therapy, or between 1 and 7 days after other antihypertensive agents had been withdrawn in patients receiving such therapy. The glomerular filtration rate (GFR) was estimated from the clearance of inulin, renal plasma flow (RPF) from the clearance of para-aminohippuric acid and plasma oncotic pressure (π_A) by membrane osmometry, using methods that have been described in detail elsewhere [2–5].

The fractional clearances (relative to freely permeant inulin) of endogenous albumin and IgG, and of exogenous uncharged dextrans of broad size distribution were used to evaluate glomerular barrier function. Serum and urine were stored at −70°C for no longer than three months prior to assay. Concentrations of albumin and IgG in serum and urine were determined by radial immunodiffusion. When the concentration of either protein fell below the detection limits of the low level plates used for assay of urine (50 and 20 μg/ml, respectively), we employed a sensitive enzyme-linked immunosorbent assay (ELISA), the lower detection limit of which is only 3 ng/ml [5]. Concentrations of dextran were assayed with anthrone after plasma and urine had been separated into narrow 2 Å fractions by gel permeation chromatography as described elsewhere [5]. To ensure reproducibility over the five-year study period, the gel columns were recalibrated at three monthly intervals using five narrowly dispersed dextran standards of varying but known molecular radius. The interstudy coefficient of variation (CV) for dextran sieving coefficients was determined by repeating the clearance, separation and assay procedures in nine members of the control group one month after the initial study. The CV was 6.4 for the 30 Å fraction and between 2.7 and 3.8% for the remaining fractions.

Analysis of intrinsic glomerular membrane parameters

The size-selective properties of the glomerular barrier were computed from dextran sieving profiles using a heteroporous representation of the glomerular capillary wall that has been described previously [7]. In this model the major portion of the capillary wall is assumed to be perforated by restrictive, cylindrical pores of identical radius (r_0). The model assumes that there exists in addition a parallel "shunt pathway" which does not discriminate on the basis of dextran size, and through which passes a small fraction of the filtrate volume. The shunt pathway is characterized by a parameter ω_0 which governs the fraction of the total filtrate volume passing through the nonrestrictive portion of the membrane. According to this model, the membrane barrier to filtration of water and uncharged macromolecules is characterized fully by the values of r_0, ω_0, and K_f; where K_f is the product of the effective hydraulic permeability and the total glomerular capillary surface area (for two kidneys). An additional membrane parameter that can be derived from K_f and r_0 is the ratio of effective pore area-to-pore length (S′/l), which is closely related to the apparent number of restrictive pores in the two kidneys (N). More precisely, $N = S'/\pi r_0^2$. Thus, if l and r_0 are nearly constant, changes in S′/l are very nearly proportional to changes in the total number of restrictive pores, N [9, 10].

To take the effect of GFR determinants on convective and diffusive transmembrane transport into account, the model requires knowledge of GFR, RPF, π_A and the transcapillary hydraulic pressure difference, ΔP [7, 9]. The latter quantity cannot be determined directly in humans. However, using an indirect curve fitting technique, we have estimated that ΔP approximates 35 mm Hg in the healthy human kidney, and have assigned this value to the control group in the present study [11, 12]. Micropuncture determinations in insulin-treated diabetic rats have revealed moderate hyperglycemia to be associated with glomerular capillary hypertension and elevation of ΔP [13, 14]. We accordingly assigned two levels of ΔP to the diabetic groups. A lower level of 35 mm Hg is intended to cover the possibility that ΔP fails to become elevated in diabetic humans, while an upper level of 40 mm Hg is intended to simulate the situation in the diabetic rat.

Statistical analysis

Differences between groups were evaluated by ANOVA and Duncan's test for multiple comparisons using the CLINFO system. Univariate linear regression analysis was used to explore associations between the measured variables. The distribution of protein excretion rates, fractional protein clearances and membrane parameters (other than r_0) was skewed, and these values were log-transformed before analysis. All values are presented as the mean ± 1 standard error. Intergroup mean values that do not differ significantly by Duncan's multiple comparison test in Tables 1 to 5 are designated by the same letters.

Table 1. Filtration dynamics

	Control	Diabetic glomerulopathy	
		Group 1	Group 2
GFR *ml/min/1.73 m²*	108 ± 3[a]	90 ± 11[a]	70 ± 7[b]
RPF *ml/min/1.73 m²*	582 ± 24[a]	554 ± 44[a]	602 ± 52[a]
Filtration fraction	0.19 ± 0.01[a]	0.17 ± 0.03[a]	0.10 ± 0.02[b]
Afferent oncotic pressure *mm Hg*	23.0 ± 0.4[a]	23.2 ± 1.0[a]	21.4 ± 0.9[a]
Mean arterial pressure *mm Hg*	85 ± 2[a]	96 ± 3[b]	103 ± 4[b]

Means in this and subsequent tables that are not different from one another by Duncan's multiple comparison test are designated with the same letter.

Table 2. Renal protein handling

	Control	Diabetic glomerulopathy	
		Group 1	Group 2
Excretion rate *μg/min*			
Albumin	13 ± 3	140 ± 20	2376 ± 367
IgG	1.7 ± 0.3[a]	17 ± 4[b]	205 ± 41[c]
Serum concentration *mg/dl*			
Albumin	3860 ± 91[a]	3871 ± 127[a]	3342 ± 146[b]
IgG	946 ± 74[a]	1090 ± 98[a]	877 ± 74[a]
Fractional clearance ($\times 10^{-5}$)			
Albumin	0.35 ± 0.10[a]	5.8 ± 1.3[b]	141 ± 33[c]
IgG	0.14 ± 0.03[a]	2.6 ± 0.9[b]	62 ± 21[c]
IgG-to-albumin clearance ratio *selectivity index*	0.57 ± 0.15[a]	0.60 ± 0.15[a]	0.31 ± 0.04[a]

See Table 1 legend for description of data presentation.

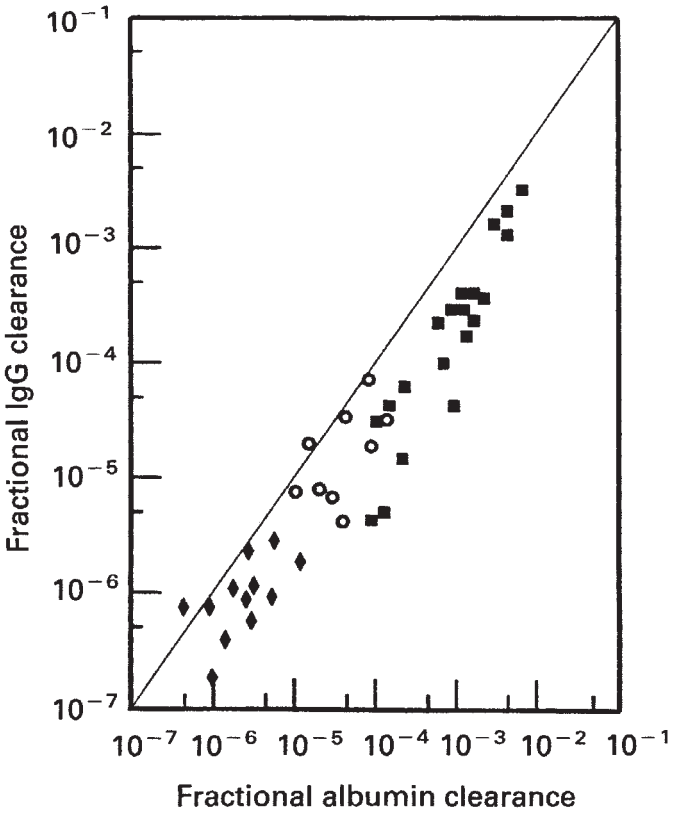

Fig. 1. *Relationship between the fractional clearances of IgG and albumin in control and diabetic subjects.* Symbols for control and diabetic categories in this and subsequent figures are: control (◆), group 1 (○) and group 2 (■). Some control subjects and 2 members of the diabetic Group 1 were studied before the ELISA technique for determining urinary albumin and IgG concentration was available, with the result that the regression analysis does not include all members of these 2 groups. $r = 0.95$; $P < 0.0001$.

Results

Filtration dynamics

Despite an absence of azotemia, GFR was significantly depressed by 35% in diabetic Group 2 (Table 1). The RPF remained within the normal range however, with the result that the filtration fraction was also markedly depressed. Neither GFR, RPF nor the filtration fraction in diabetic Group 1 with microalbuminuria differed from corresponding control values. Whereas plasma oncotic pressure tended to be slightly depressed in diabetic Group 2 only, the arterial pressure was significantly elevated in both diabetic groups, exceeding the control value by 11 in Group 1 and by 18 mm Hg in Group 2, on average.

Renal protein handling

The incremental albumin excretion rate according to which the diabetic patients were grouped was accompanied by a parallel increase in the urinary IgG excretion rate (Table 2). Urinary losses of albumin in Group 2 but not Group 1 were sufficiently heavy to lower the level of circulating albumin in serum. The corresponding serum IgG level was numerically but not significantly lowered.

The fractional clearances of albumin and IgG varied in parallel with, but were proportionately more elevated than corresponding protein excretion rates in the two diabetic groups (Table 2). The relationship between the fractional clearances of each protein is illustrated in Figure 1. As shown the fractional clearance of albumin was highly correlated with that of IgG over the range of values observed in the control and diabetic groups; by linear regression analysis the coefficient of correlation was 0.95 ($P < 0.0001$). Figure 1 also shows that fractional albumin clearance exceeded that of IgG with only rare exceptions. Surprisingly, the proteinuria tended to be most selective in diabetic Group 2, with the selectivity index (IgG-to-albumin clearance ratio) averaging only 0.31 ± 0.04 compared to 0.60 ± 0.15 and 0.57 ± 0.15 in diabetic Group 1 and controls, respectively (Table 2).

Transglomerular dextran transport

Like inulin, dextran fails to undergo either measurable tubular reabsorption or secretion [10]. It follows that dividing the clearance of a given dextran by that of inulin corrects for water reabsorption along the nephron, and thus provides a measure of the sieving coefficient (Bowman's space-to-plasma concentration ratio) for that dextran. The sieving coefficients for dextrans of graded size are summarized in Table 3, and the mean dextran sieving profiles for each group are illustrated in Figure 2. The configuration of the sieving profiles in the two diabetic groups was similarly altered from that in controls. The low radius ends of the diabetic profiles were significantly depressed. Each

Table 3. Fractional dextran clearances

	30Å	34Å	38Å	42Å	46Å	50Å	54Å	58Å
Control	0.7158[a] ±0.0247	0.4162[a] ±0.0115	0.2191[a] ±0.0068	0.1077[a] ±0.0036	0.0485[a] ±0.0020	0.0210[a] ±0.0012	0.0089[a] ±0.0006	0.0040[a] ±0.0004
Diabetic glomerulopathy								
Group 1	0.5757[b] ±0.0454	0.3338[b] ±0.0197	0.1710[b] ±0.0151	0.0870[b] ±0.0111	0.0434[a] ±0.0079	0.0217[a] ±0.0054	0.0108[a] ±0.0033	0.0059[a,b] ±0.0022
Group 2	0.5321[b] ±0.0290	0.3001[b] ±0.0145	0.1611[b] ±0.0081	0.0842[b] ±0.0050	0.0430[a] ±0.0031	0.0225[a] ±0.0021	0.0124[a] ±0.0014	0.0077[b] ±0.0011

See Table 1 legend for description of data presentation.

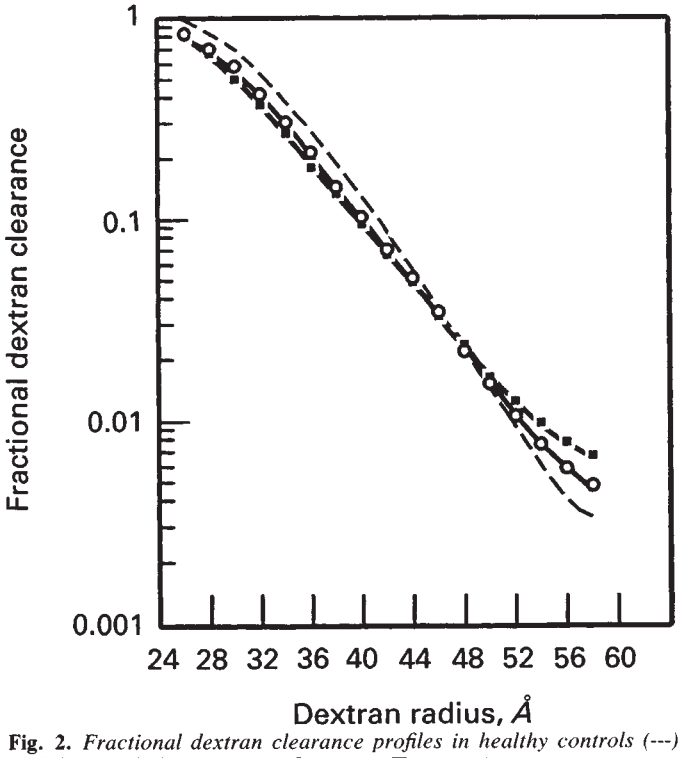

Fig. 2. *Fractional dextran clearance profiles in healthy controls (---) and the two diabetic groups (○, micro; ■, macro).*

diabetic sieving profile then intersected the corresponding control profile at between 47 and 48 Å. Beyond the intersection the passage of large, nearly impermeant dextrans was enhanced, although only the elevation of the sieving coefficients for the two largest dextran fractions (r_s = 58 and 60 Å) in diabetic Group 2 reached statistical significance (Table 3). Nevertheless, the trend to selective elevation of sieving coefficients of large, nearly impermeant dextrans points to a membrane with a less sharp cut-off in the diabetic groups than in controls.

Analysis of membrane parameters

The intrinsic membrane properties of the members of each group were computed by applying the dextran sieving profiles, GFR, RPF, π_A and the assumed levels of ΔP to the "isoporous plus shunt" membrane model [7]. The computed range for each membrane parameter is provided in Table 4. A graded reduction for K_f and S'/l is evident, such that the magnitude of ultrafiltration capacity of all filtering glomerular capillaries in the two human kidneys is control > diabetic Group 1 > diabetic Group 2. Notice that a 5 mm Hg elevation of ΔP has a marked effect on computed K_f and S'/l, lowering these two quantities by between 25 and 40%. Notice also that although the range of computed values for K_f and S'/l in microalbuminuric diabetic Group 1 does not overlap the estimated control values, depression of either the ultrafiltration coefficient (K_f) or pore density (S'/l) does not reach significance unless ΔP is elevated to 40 mm Hg (Table 4). In contrast each measure is significantly depressed below the corresponding control value in diabetic Group 2, regardless of whether or not ΔP is elevated.

In contrast to K_f and S'/l, the computed values for the two pore parameters are only slightly influenced by a 5 mm Hg increase in ΔP (Table 4). The radius of restrictive pores (r_0) is below control by approximately 1 Å in each diabetic group. The opposite is true of ω_0, the parameter governing the non-discriminatory pores. The shunt pathway, as reflected by ω_0, is three to four times more prominent in Group 2 diabetics than in controls (4.0 to 4.8 vs. 1.3×10^{-3}). Once again the corresponding value for Group 1 is intermediate (1.6 to 2.3×10^{-3}). However, as for K_f and S'/l, the excess of ω_0 over control only reaches statistical significance in the event that ΔP is elevated in these microalbuminuric subjects.

Given that the higher arterial pressure in diabetic subjects is likely to be transmitted into glomerular capillaries, we will assume that ΔP = 40 mm Hg in diabetic Groups 1 and 2 in an attempt to explore further the relationship between proteinuria and shunt-like pores.

That both the development and magnitude of proteinuria in DGD is related to the prominence of the shunt pathway is indicated by a strong relationship between fractional albumin or IgG clearance and ω_0 among all subjects studied (Fig. 3). In an effort to determine whether the shunt pathway can account for the observed fractional protein clearances, we have computed the clearance of a hypothetical macromolecule that is attributable to the shunt pathway (Table 5). This quantity is denoted Θ_∞ and neglects tubular reabsorption [7]. Because the luminal concentration of a macromolecule will increase with distance along the glomerular capillaries as water is removed by ultrafiltration, Θ_∞ slightly exceeds ω_0. As shown in Figure 4, Θ_∞ exceeds the respective fractional clearances of albumin and IgG in each group, suggesting that transmembrane shunting of plasma is always sufficient to account for the observed magnitude of the fractional protein clearances. We note with interest, however, that the trend for Θ_∞ in diabetic Group 1 to exceed control does not reach statistical significance, and that Θ_∞ is significantly enhanced only in Group 2 (Table 5). Also noteworthy is the disparate relationship between Θ_∞ and the fractional

Table 4. Membrane parameters

	Control	Diabetic glomerulopathy			
		Group 1		Group 2	
ΔP *mm Hg*	35	35	(40)	35	(40)
K_f *ml/min · mmHg/1.73 m²*	16.2 ± 2.0[a]	12.5 ± 2.5[a]	(7.4 ± 1.0[b])	9.0 ± 2.5[b]	(5.1 ± 0.7[c])
S'/l km	349 ± 43[a]	282 ± 53[a]	(165 ± 22[b])	214 ± 65[b]	(114 ± 17[c])
r_0 *Å*	56.9 ± 0.2[a]	55.4 ± 0.6[b]	(56.0 ± 0.7)[a]	55.6 ± 0.5[b]	(56.0 ± 0.4[a])
$\omega_0 \times 10^{-3}$	1.3 ± 0.1[a]	1.6 ± 0.3[a]	(2.3 ± 0.5[b])	4.0 ± 0.7[b]	(4.8 ± 0.8[c])

See Table 1 legend for description of data presentation.

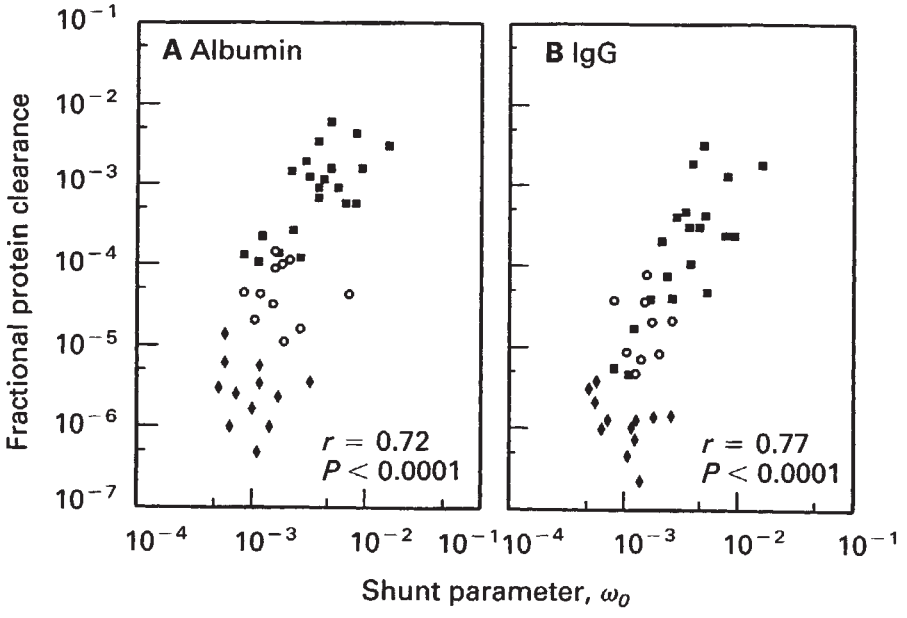

Fig. 3. *Relationship between the fractional urinary clearances of albumin* (**A**) *and IgG* (**B**), *and the corresponding shunt parameter* (ω_0) *in control* (◆) *and diabetic subjects* (○, *micro;* ■, *macro*), *assuming an elevated* ΔP *of 40 mm Hg in the diabetic subjects.* The regression equations are y = 1.97 + 2.29 x and y = 2.02 + 2.48 x for albumin and IgG, respectively. If ΔP remains 35 mm Hg in the diabetic subjects, the regression equations and correlation coefficients are y = 1.95 + 2.22 x, r = 0.66, and y = 1.80 + 2.32 x, r = 0.70, for albumin and IgG, respectively. The explanation for the reduced number of control and Group 1 comparisons is given in the legend to Figure 1.

Table 5. Hypothetical fractional clearance (via shunt) and reabsorption

	Control	Group 1	Group 2
$\Theta_\infty \times 10^{-5}$	416 ± 42[a]	544 ± 166[a]	769 ± 108[b]
Θ alb/Θ_∞	0.0009 ± 0.0003[a]	0.016 ± 0.004[b]	0.22 ± 0.06[c]
Θ IgG/Θ_∞	0.0004 ± 0.0001[a]	0.009 ± 0.003[b]	0.10 ± 0.04[c]

See Table 1 legend for description of data presentation.

protein clearances. Whereas Θ_∞ exceeds the respective fractional clearances of albumin and IgG by five- and tenfold in diabetic Group 2, the corresponding excess in diabetic Group 1 and controls is by 2 and 3 orders of magnitude respectively (Table 5, Fig. 4). This suggests that factors other than or additional to transmembrane shunting of albumin and IgG may be implicated in the differences in the magnitude of proteinuria between patients with microalbuminuria and those with macroalbuminuria.

Discussion

Although cross sectional in nature, our theoretical analysis of membrane pore structure suggests that evolution of DGD through a microalbuminuric stage to one of overt proteinuria represents a continuum of progressively deranged glomerular capillary wall function. This derangement is characterized by a progressive reduction in the number of glomerular pores ($\downarrow$ S'/l) accompanied by a shift in pore-size distribution towards pores of larger radius ($\uparrow \omega_0$). A progressive reduction in pore number likely provides the basis for the irrevocable decline in GFR that typifies DGD, and is consistent with morphometric analyses of glomerular structure, which suggest an incremental lowering of glomerular capillary filtration surface area as DGD advances [15, 16]. Although its structural basis is unknown, the growing fraction of glomerular pores which behave as shunts could provide a basis for increasing proteinuria as DGD progresses from the micro- to the macroalbuminuric stage of injury. In keeping with this possibility we have shown a strong correlation between the magnitude of the shunted fraction of filtrate volume (ω_0) and that of fractional clearances of albumin and IgG in the subjects of the present study (Fig. 3). That transmembrane shunting accounts exclusively for the magnitude and composition of proteinuria in early DGD cannot be inferred from the present findings, however.

One of the findings that casts doubt upon shunt-like, barrier defects as an exclusive mechanism for proteinuria is the excess of albumin over IgG clearance over the entire range of barrier dysfunction observed in the present study. Were pores that fail to restrict dextrans of r_s = 60 Å also nondiscriminating towards albumin and IgG, and the sole portal of entry for each protein into Bowman's Space fluid, one would expect equality of the respective fractional protein clearances. On the other hand, the enhancement of albumin over IgG clearances could be explained by a dual route of transcapillary albumin escape. Judged

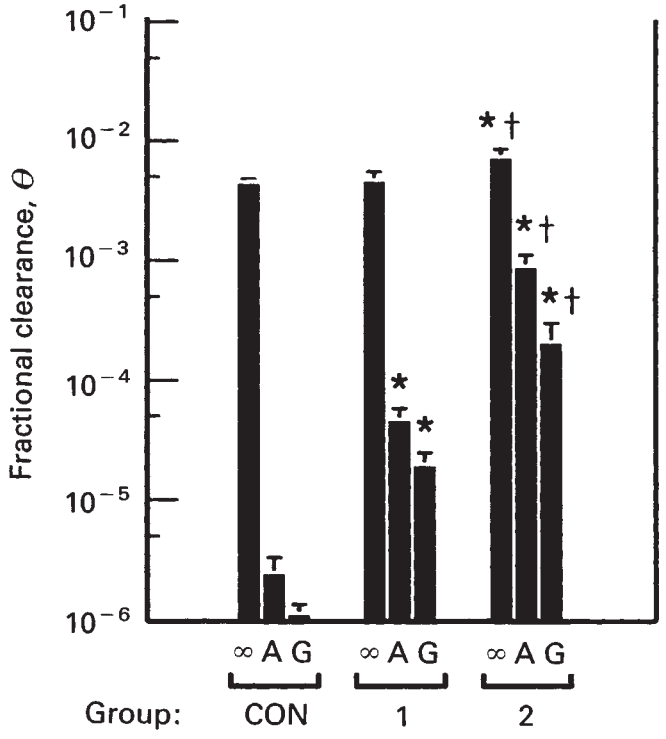

Fig. 4. *Fractional clearance attributable to the shunt pathway (Θ_∞) in each group (assuming ΔP is 40 mm Hg in the diabetic groups), and the corresponding fractional urinary clearances of albumin (A) and IgG (G).* $^*P < 0.05$ vs. control; $^\dagger P < 0.05$ vs. group 1.

by its molecular radius, it is conceivable that albumin (r_s = 36 Å) can permeate the restrictive pores of the major component of the membrane (r_0 = 56 Å), whereas IgG (r_s = 55 Å) cannot. Thus, loss of fixed, negatively-charged sites from the restrictive portion of the membrane in DGD could impair electrostatic retardation, and in this way provide a second pathway for albumin filtration which is inaccessible to IgG [17]. Yet another explanation for disproportionate clearance of albumin is that the predominantly neutral or cationic molecules of filtered IgG bind more avidly to the negatively-charged glycocalyx of proximal tubular brush border than do the anionic molecules of filtered albumin [18, 19]. Facilitated endocytosis of IgG by proximal tubular cells could then permit a disproportionate fraction of the filtered albumin load to reach final urine.

That proteinuria in DGD may not be uniquely attributable to shunt-like glomerular pores in DGD is also suggested by our computations for Θ_∞, the fractional clearance of any large, non-reabsorbed molecule, which can be ascribed to the postulated shunts. As noted already in connection with Figure 4, the graded increases above control in fractional protein clearance of diabetic Groups 1 and 2 are proportionately much larger than the calculated increases in Θ_∞. Stated another way the values of Θ_∞ suggest that normal individuals and diabetic patients with microalbuminuria should be almost as heavily proteinuric as the macroalbuminuric diabetic patients of Group 2, yet of course they are not.

Tubular reabsorption of a variable fraction of filtered protein among the groups may contribute to but does not appear to account completely for the discrepancy, however. Fractional protein reabsorption increases as the filtered load diminishes [20, 21]. In keeping with the observed disparity in the three study groups, this should lower the urinary fractional clearance of proteins by the greatest percentage in controls, by an intermediate percentage in diabetic Group 1, and by the smallest percentage in diabetic Group 2. However, dividing fractional protein clearances by Θ_∞ suggests that 99% or more of the shunted protein load would have to be reabsorbed in controls and patients of diabetic Group 1, if this were the sole factor (Table 5). Such fractional reabsorptive rates are greatly in excess of corresponding rates determined directly by micropuncture in non-proteinuric rats [20, 21]. To the extent that fractional protein reabsorption in the human subjects of the present study resembles that in the rat, we infer that the corresponding fractional reabsorption of protein would be no greater than 90% in the members of the control group and those of diabetic Group 1.

An attractive explanation for the remaining discrepancy between Θ_∞ and fractional protein clearances relates to the fact that random-coil polymers such as dextran do not behave as the ideal, neutral spheres assumed in the pore size calculations. This has been demonstrated both in vitro by diffusion experiments using synthetic membranes of uniform and independently measured pore size [22, 23] and in vivo in the rat glomerulus [24, 25]. In both circumstances the transmembrane passage of uncharged dextrans has been shown to be more rapid than that of uncharged, spherical molecules (ficolls or proteins) of equivalent Stokes-Einstein radius. Thus, applying the conventional solid-sphere model to dextran data will tend to overestimate the membrane permeability to proteins. In particular much or all of the transport of the largest dextrans in controls, which the present model attributes to the shunt, may in fact represent the unusually rapid passage of dextrans through a small-pore component of the membrane, one which permits little or no passage of albumin or IgG. According to this reasoning the values of Θ_∞ in all groups might be overestimated by roughly similar amounts. It follows that the actual value of Θ_∞ in the two diabetic groups could be smaller than those estimated in Table 5 and perhaps, sufficient only to account for the observed fractional clearance of IgG, but not that of albumin.

A more quantitative test of this hypothesis must await the development of more realistic models for hindered transport of random-coil polymers. If confirmed, however, it would point away from shunt-like pores as a sole explanation for micro- or macroalbuminuria in early DGD. A concomitant increase in albumin passage through restrictive pores which are charge-depleted could then be invoked to explain the predominance of urinary albumin over IgG clearance in this disorder.

Acknowledgments

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