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Proteinuria and functional characteristics of the glomerular barrier in diabetic nephropathy

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Proteinuria and functional characteristics of the glomerular barrier in diabetic nephropathy. Fractional clearances of uncharged dextran 40 and anionic proteins were performed in an attempt to elucidate the defect in glomerular barrier function responsible for heavy proteinuria in diabetic nephropathy. Notwithstanding urinary albumin excretion ($U_{alb}V$) at 3634 ± 608 μg/min, the fractional clearance for dextran molecules with Einstein-Stokes radii (r) between 22 and 36 Å was depressed in 12 patients with advanced diabetic nephropathy, which suggests a reduction in mean glomerular pore size or density. Equivalent restriction to transglomerular passage of dextrans with a $r < 36$ Å in 7 patients with minimal change nephropathy was associated with a similarly enhanced proteinuria ($U_{alb}V$, 3333 ± 759 μg/min). The dissociation between fractional clearances for neutral and anionic macromolecules in both disorders is consistent with loss of glomerular electrostatic charge. In diabetic nephropathy, however, the fractional clearances for large dextrans and test proteins considerably exceeded corresponding values in minimal change nephropathy when $r \geq 36$ Å. Furthermore, the fractional clearances for test proteins were two orders of magnitude smaller than that for equivalent-sized dextrans in minimal change nephropathy, whereas this difference was much less in diabetic nephropathy. Thus, a selective increase in transglomerular passage of large molecules and a progressive loss of ability to discriminate between large molecules of different configuration distinguish the glomerular capillary wall in diabetic nephropathy from that in minimal change nephropathy.

Protéinurie et caractéristiques fonctionnelles de la barrière glomérulaire dans la néphropathie diabétique. Les clearances fractionnelles de dextran 40 neutre et de protéines anioniques ont été mesurées afin d'étudier le défaut de la fonction de la barrière glomérulaire responsable de la protéinurie massive au cours de la néphropathie diabétique. Malgré une excrétion urinaire d'albumine ($U_{alb}V$) de 3634 ± 608 ug/min, la clearance fractionnelle pour les molécules de dextran dont le rayon d'Einstein-Stokes (r) était compris entre 22 et 36 Å était abaissé chez 12 malades atteints de néphropathie diabétique avancée, ce qui suggère une diminution de la taille moyenne ou de la densité des pores. Une diminution équivalente de la restriction au franchissement du glomérule de dextran $r < 36$ Å chez 7 malades atteints de néphropathie à modifications minimes était associée à une protéinurie du même ordre ($U_{alb}V$, 3333 ± 759 μg/min). La dissociation entre les clearances fractionnelles des macromolécules neutres et anioniques dans les deux affections est compatible avec une perte des charges électrostatiques glomérulaires. Dans la néphropathie diabétique, cependant les clearances fractionnelles des dextrans de grande taille et des protéines étudiées était plus élevé que les valeurs obtenues dans les néphropathies à modifications minimes quand $r \geq 36$ Å. De plus, les clearances fractionnelles pour les protéines étudiées sont plus es petit de deux ordres de grandeur que les clearances fractionnelles pour les dextrans de taille équivalente dans la néphropathie à modifications minimes, alors que cette différence était bien moindre dans la néphropathie diabétique. Ainsi une augmentation sélective du passage transglomérulaire des grosses molécules et une perte progressive de la capacité de discriminer entre les grosses molécules selon leur configuration différencie la paroi du capillaire glomérulaire de la néphropathie diabétique de celle de la néphropathie à modifications minimes.

Heavy proteinuria is the clinical hallmark of diabetic nephropathy. It is associated with *(1)* long-standing, insulin-requiring diabetes, usually of more than 10 years' duration [1–4], *(2)* depression of the rate of glomerular ultrafiltration (GFR) [4, 5], *(3)* advanced diabetic glomerulosclerosis, invariably of the diffuse and sometimes also of the nodular type [6–9], and *(4)* abnormal biochemical composition of isolated glomeruli characterized by an increase in hydroxylysine-disaccharide-linked residues [10].

From a functional standpoint, the glomerular capillary wall generally has been viewed as a porous molecular sieve capable of permitting essentially free filtration of water and "small" plasma solutes while restricting the passage of large molecules including plasma proteins. According to this view, emphasized initially by Pappenheimer, Renkin, and Borrero [11] and Landis and Pappenheimer [12], restriction to transport of certain molecules across a limiting barrier is based on the assumption that the barrier is perforated by slits or cylindrical pores. With fractional clearances of macromolecules, it has been possible to calculate an average pore radius of approximately 52 Å for the glomerular capillary wall in both normal humans [13, 14] and Mu-

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nich-Wistar rats [15, 16]. Although no such pores can be seen on electron microscopy, the localization of electron-dense tracers beneath the glomerular basement membrane and at the filtration slit diaphragms has led workers to propose that these structures constitute the major barriers to the filtration of macromolecules in the three-layered glomerular capillary wall [17, 19].

Given the profound structural, functional, and biochemical alterations in the glomerular capillary wall in diabetic nephropathy, it has been postulated that an increase in effective pore size permits the escape of plasma proteins into the urine in this disorder [20–22]. Recent studies of human and experimental glomerular diseases, however, have demonstrated that proteinuria can be attributed to alterations in properties of the glomerular filter other than those related to pore size [23–25]. In these glomerular diseases, proteinuria and enhanced clearance of anionic macromolecules have been found in the presence of a reduction in mean pore size or density, suggesting that a key alteration is a deficiency in electrostatic repulsion of negatively charged macromolecules by the diseased glomerular capillary wall.

Accordingly, we attempted to define the functional characteristics of the glomerular barrier in patients with diabetic nephropathy by performing fractional clearances with three test macromolecules that differ with respect to size, charge, and shape. They were *(1)* dextran 40 (Rheomacrodex, Pharmacia Fine Chemicals AB), a series of uncharged and loosely coiled polymers, the component molecules of which have Einstein-Stokes radii (r) varying between 20 and 57 Å; *(2)* endogenous albumin (alb), a globular protein with a well-defined molecular size (r = 36 Å) and charge (isoelectric point, 4.8); and *(3)* endogenous immunoglobulin G (IgG), a larger protein (r = 50 Å), the component subclasses of which have isoelectric points varying between 6.4 and 7.2.

Methods

Study population. Twelve patients with diabetic nephropathy and 19 controls were studied. The study protocol and informed consent procedure were approved by the Stanford University Committee for the Protection of Human Subjects in Research.

Experimental group. This group consisted of 12 patients with diabetes who had proteinuria ranging from 2.5 to 12 g/day. The criterion for acceptance into the experimental group was the opthalmoscopic evidence of diabetic retinopathy in a patient with heavy proteinuria (> 2 g/day) because the almost invariable coexistence of diabetic retinopathy with diabetic glomerulosclerosis permits the diagnosis of diabetic nephropathy to be made without resort to renal biopsy [26–28]. The 8 males and 4 females in this group varied in age from 32 to 60 years.

Control groups. The controls comprised three groups, 12 males and 7 females, whose ages (between 18 and 63 years) were similar to those in the diabetic experimental group. *Group 1.* This group was composed of 7 healthy volunteers who were devoid of renal disease and hypertension as judged by a negative history, clinical examination, and urinalysis. *Group 2.* Five nonnephrotic patients with advanced but stable chronic renal diseases (CRD) were selected for study because their GFR's were depressed over a range similar to that in diabetic patients (8 to 60 ml/min/1.73 m^2). Their urinary protein excretion rates were modest, amounting to < 2 g/day. Their underlying renal diseases included polycystic kidney disease (N = 2), hypertension with nephrosclerosis (N = 1), chronic tubulointerstial disease (N = 1), and chronic lupus nephritis (N = 1). The latter two diagnoses were confirmed by renal biopsy. *Group 3.* This group comprised 7 patients in whom a nephrotic syndrome was associated with biopsy-proven minimal change nephropathy (MCN). This discreet variety of the nephrotic syndrome was selected for study because, unlike diabetic nephropathy, glomerular structure is only minimally and reversibly altered.

Study protocol. Each subject was required to empty his or her bladder, by voiding, before receiving an i.v. injection containing inulin (60 mg/kg), PAH (36 mg/kg), and dextran 40 (130 mg/kg). Following this injection, a high rate of urine flow was induced by oral water loading. At the end of a 40- to 60-min equilibration period, their bladders were again emptied by voiding; then, three to five carefully timed urine collections were made. Venous blood was sampled at 5, 10, 20, and 40 min following the test solute infusion and thereafter at the beginning and end of each urine collection.

Inulin and PAH clearances were calculated from the average clearance value or all timed collection periods by using the equation

$$C_s = (U/P)_s \cdot V/1.73\ m^2 \qquad (1)$$

where $(U/P)_s$ is the urine/plasma solute (inulin and

PAH) concentration ratio, and V is the urine flow rate expressed in milliliters per minute. P_s, the mid-collection-point plasma solute concentration, was calculated from the plasma inulin and PAH concentration curves [29].

The fractional clearance (θ) was determined for each of the three test macromolecules (M), with inulin (In) as the reference solute, from the equation

$$\theta_M = (U/P)_M/(U/P)_{In} \quad (2)$$

where $(U/P)_M$ is the urine/plasma concentration ratio for each test macromolecule, and $(U/P)_{In}$ were determined from urine and plasma from the initial collection period. The urinary albumin ($U_{alb}V$) and IgG ($U_{IgG}V$) excretion rates (expressed in micrograms per minute) were calculated from the product of the respective urinary concentration of each protein (expressed in milligrams per deciliter) and the urine flow rate (expressed in milliliters per minute).

Laboratory determinations. For the calculation of GFR, the inulin concentration in urine and plasma from each collection period was determined by the autoanalyzer method of Fjelbo and Stamey [30]. This method uses the fructose-specific reagent resorcinol, and we have found it to be uninfluenced by the presence of dextran. The autoanalyzer method of Harvey and Brothers, which uses Marshall reagent, was used for the determination of PAH concentrations [31]. Following gel permeation chromatography of plasma and urine, eluted fractions were assayed for dextran and inulin concentration by a modification of the autoanalyzer Anthrone method of Scott and Melvin [32]. Dextran standards were prepared for assaying the early dextran-containing fractions (nos. 30 to 45). Separate inulin standards were used for assay of the smaller inulin molecules (r = 11 to 17 Å) that appeared in late eluted fractions (nos. 48 to 57).

Albumin and IgG concentrations in plasma and urine were determined by radial immunodiffusion (Kallestad Labs, Inc.) [33]. Test protein concentrations in unconcentrated urine were determined by using low-level immunodiffusion plates (Endoplates albumin or IgG cerebrospinal fluid test kit, Kallestad Labs, Inc.). These plates permit the measurement of these proteins over a concentration range of 4 to 100 mg/dl for albumin and 1 to 25 mg/dl for IgG. To ensure that heavy chain-containing degradation products of IgG did not spuriously elevate urinary IgG concentration, we tested 25- to 100-fold concentrated urine samples from 5 patients with diabetic nephropathy against heavy- and light-chain antisera by the double-diffusion Ouchterlony technique and by immunoelectrophoresis [33]. Plasma oncotic pressure (π) was determined with a Weil oncometer (Instrumentation Laboratories, model IL-186).

Chromatographic procedures. Separation of dextran 40 in plasma and urine into narrow fractions (approximately 2 Å) was accomplished by gel permeation chromatography with Sephacryl S200. Two columns, 91 and 95 cm in length, with internal diameters of 1.6 cm, were used. Each column was calibrated with three narrow dextran fractions of known molecular size (provided by Pharmacia Fine Chemicals AB, Uppsula, Sweden). Using 0.3% saline as eluant, we collected 2.6 ml of eluted fractions with an automatic fractionator (Gilson). The void volume (V_o) was determined with blue dextran, and the fractional volume available to the solute (K_{av}) was then calculated as

$$K_{av} = (V_e - V_o)/(V_t - V_o) \quad (3)$$

where V_e is the elution volume of the solute, and V_t is the total volume of the gel column [34]. Stokes-Einstein radii (r) for individual dextran fractions were calculated from K_{av} [35].

Statistical methods. All results are expressed as the means ± SEM. Student's *t* test for unpaired data was used to evaluate differences between the experimental and control groups.

Results

Clinical and laboratory findings. The onset of diabetes mellitus preceded the study by 6 to 33 years in the diabetic patients, 10 of whom were requiring insulin, and 2 of whom were receiving oral hypoglycemic agents. Pertinent laboratory findings for these patients are summarized and compared with those of the control groups in Table 1. Inulin and PAH clearances were depressed below 30 and 150 ml/min/1.73 m^2, respectively, in all but 2 diabetic patients, and were significantly lower, on average, than the corresponding values in normal volunteers and in patients with MCN. Mean inulin (18 ± 5 ml/min/1.73 m^2) and PAH clearances (80 ± 24 ml/min/1.73 m^2) in diabetic patients were also somewhat lower than they were in CRD control patients, but these differences did not reach statistical significance.

Mean $U_{alb}V$ in patients with diabetic nephropathy was similar to that in nephrotic patients with MCN (3634 ± 608 vs. 3333 ± 759 μg/min) but was more than threefold larger than in patients with CRD. Mean $U_{IgG}V$, which averaged 596 ± 132 μg/

min in diabetic nephropathy, was elevated ninefold and sixfold, respectively, above the corresponding values in patients with MCN and CRD.

Fractional dextran clearances (Fig. 1 and Table 2). The fractional clearance values of dextran 40 relative to inulin (θ_D) plotted as a function of the Einstein-Stokes radius (r) are illustrated for all four study groups in Fig. 1. The results of normal control subjects (Fig. 1a and Table 2) are similar to those reported by others in man [14, 36]. Measureable restriction to dextran indicated by θ_D falling below unity occurred when r > 22 Å. With increasing molecular size, θ_D declined and approached zero for dextrans with r > 48 Å. Despite massive proteinuria, the mean fractional dextran clearance profile in diabetic nephropathy was depressed below that of normal controls in the dextran r interval 24 to 36 Å (Fig. 1a and Table 2). θ_D was also depressed relative to control patients with CRD, the depression being evident over the entire range of molecular sizes examined (r = 22 to 48 Å, Fig. 1b). Although θ_D for dextran molecules with r < 36 Å was similarly depressed in patients with diabetic nephropathy and in those with minimal change nephropathy, θ_D for larger molecules was significantly higher in diabetic than in minimal change nephropathy patients. (Fig. 1c and Table 2).

Fractional protein clearances. To permit comparison between glomerular proteinuria and the glomerular filtration of dextrans, we calculated fractional protein clearances for patients with diabetic nephropathy and for nephrotic controls with MCN. The accuracy of this calculation requires that heavy-chain reactivity measured by radial immunodiffusion reflects intact IgG. In urine samples from 5 diabetic patients, immunoelectrophoresis revealed all detectable light and heavy chains of IgG to have the same electrophoretic mobility as intact plasma IgG. Furthermore, reactions of identity were obtained with isolated polyclonal human IgG when the urinary IgG was examined by the Ouchterlony double-diffusion technique of using antihuman antibodies specific for IgG heavy chains and for kappa and lambda light chains (Meloy Labs, Inc.). Thus, IgG fragments were not detectable by either method in urine samples from these diabetic patients. These findings are in keeping with the more quantitative data of Petersen et al, who used gel permeation chromatography combined with immunodiffusion. They found intact urinary IgG and Fc fragments to account for 98.8 and 1.2%, respectively, of immunoreactivity to a heavy-chain-specific IgG antibody in patients with glomerular proteinuria [37].

The relationship between fractional clearance of test proteins and dextrans of equivalent size is illustrated in Fig. 2. The θ_{alb} in patients with minimal change nephropathy averaged $1.75 \pm 0.59 \times 10^{-3}$, a value 110 times smaller than that for θ_{D36} (0.19 ± 0.02). Similarly, θ_{IgG}, which averaged $1.3 \pm 0.4 \times 10^{-4}$, was 230 times less than θ_D for a dextran molecule with r = 48 Å, which averaged 0.03 ± 0.01. In patients with diabetic nephropathy, θ_{alb} and θ_{IgG} were one order of magnitude larger than the corresponding values in MCN, averaging $1.305 \pm 0.270 \times 10^{-2}$ and $6.43 \pm 1.47 \times 10^{-3}$, respectively. Thus, the discrepancy between the fractional clearances of the two test species was much less in diabetic than it was in minimal change nephropathy.

Discussion

Although proteinuria seldom appears before 10 years have elapsed from the onset of juvenile diabetes mellitus, quantitative electron microscopy has revealed thickening of the GBM within 5 years [38–40]. To determine whether glomerular permselectivity to macromolecules was altered at this early stage, Mogensen studied a group of patients with juvenile diabetes, the duration of which was 5

Table 1. Laboratory findings in patients with diabetic nephropathy and in controls[a]

Groups	C_{In}	C_{PAH}	π	$U_{alb}V$	$U_{IgG}V$
	ml/min/1.73 m²		*mm Hg*	*μg/min*	
Diabetic nephropathy (N = 12)	18	80	20.0	3634	596
	±5	±24	±1.3	±608	±132
Control groups					
(1) Normal volunteers (N = 7)	99[b]	513[b]	27.7[b]	ND	ND
	±4	±54	±0.7		
(2) Chronic renal disease (N = 5)	31	111	27.2[b]	1076[b]	102[b]
	±11	±43	±2.5	±327	±36
(3) Minimal change nephropathy	80[b]	371[b]	17.6	3333	65[b]
(N = 7)	±8	±82	±1.8	±759	±23

[a] Values are the means ± SEM. π is plasma oncotic pressure. ND is not detectable.
[b] $P < 0.05$, compared with the diabetic nephropathy group.

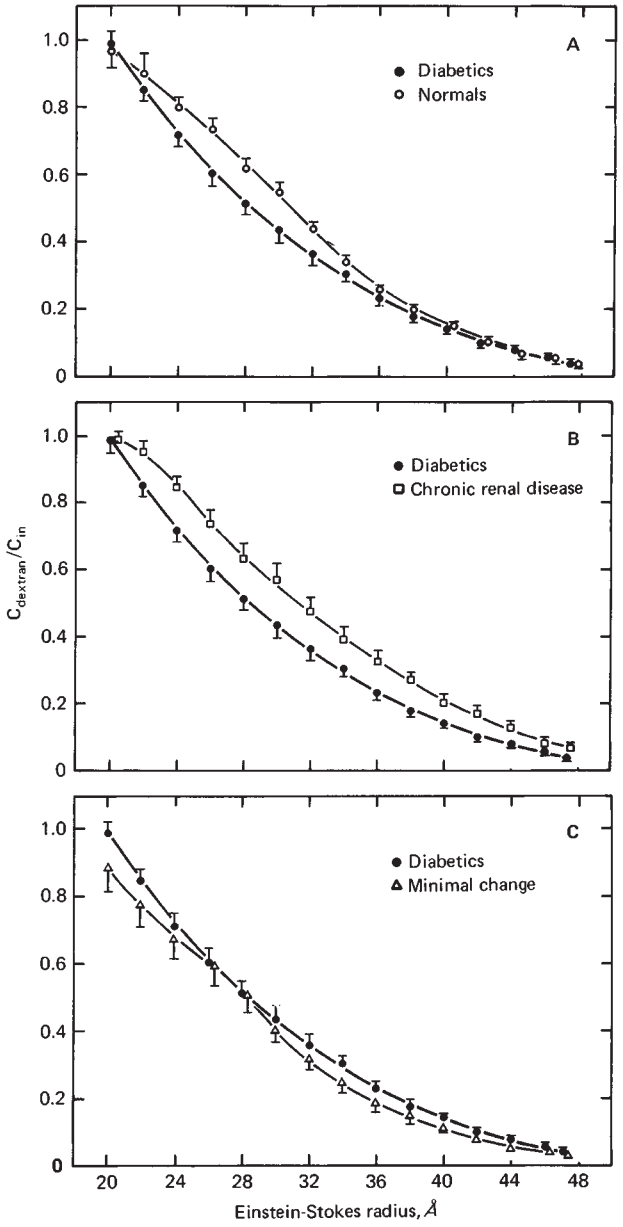

Fig. 1. *Fractional dextran clearance profile (mean ± SEM) in diabetic nephropathy* (•) *compared with: (A) normal subjects* ([○], upper panel), *(B) patients with nonnephrotic chronic renal disease* ([□], middle panel), *and (C) minimal change nephropathy* ([Δ], lower panel).

years. He found that the fractional dextran clearance profile was not different from that in a group of healthy control persons [36]. Although our data for dextran clearances confirm earlier observations that massive proteinuria persists in diabetic nephropathy even when GFR declines to very low levels [9, 41], they fail to provide evidence for an increase in average glomerular porosity in the late stages of this disorder. The clearance of PAH, and hence presumably mean glomerular plasma flow, was profoundly depressed in these diabetic patients, a finding predicted to elevate rather than depress the fractional dextran clearance profile [42]. Of the driving pressures for glomerular filtration, only plasma (afferent) oncotic pressure can be determined in man. This quantity was depressed to 72% of that in normal controls (Table 1). Although this change, by itself, is unlikely to have any measureable effect on glomerular permselectivity to macromolecules [15, 43], it might favor an increase in net glomerular ultrafiltration pressure, which could reduce the permeation of dextrans into Bowman's space. Thus, even though an alteration in glomerular capillar hemodynamics cannot be excluded as a cause for the observed depression of the fractional dextran clearance profile in diabetic nephropathy, a reduction in mean glomerular pore size or density would seem to provide a more likely explanation.

Because charged preparations of dextran are not available for use in humans, we attempted to elucidate glomerular permselectivity in our nephrotic patients by using the endogenous proteins albumin and IgG as test macromolecules. That the fractional clearances of these proteins reflect their glomerular sieving coefficients requires the assumption that tubular reabsorption is negligible or, if substantial, then nonselective in nephrotic patients. Tubular reabsorption results in less than 10% of filtered proteins appearing in the urine of normal persons, thus limiting the usefulness of fractional clearance for determining glomerular permselectivity [44]. Recent micropuncture studies in the rat, however, indicate that when the filtration of albumin is substantially increased by glomerular injury, the tubular reabsorptive mechanism rapidly becomes saturated, with the result that nearly all filtered albumin is excreted in the urine [45, 46].

Normally, the glomerular sieving coefficient for albumin is considerably lower than that of an uncharged dextran molecule with an equivalent hydrodynamic radius (36 Å). That relative restriction of albumin, a polyanion, is in part due to its negative charge has been demonstrated by the studies of Chang et al using fractional neutral dextran and dextran sulfate clearances in the normal Munich-Wistar rat [47]. There was a reduction in fractional clearance of dextran sulfate, an anionic polymer, over a range of molecular radii from 18 to 42 Å when compared to that of uncharged dextran of equivalent molecular size. The fractional clearance of dextran sulfate with an effective radius of 36 Å was 0.01 as compared to 0.19 for uncharged dextran

of equivalent size, suggesting that molecular charge is an important determinant of glomerular permselectivity to macromolecules and that electrostatic interaction between anionic molecules and the negatively charged glomerular capillary wall may be an important feature of the permselective properties of the glomerular capillary wall.

Proteins also differ in shape from dextrans of equivalent size. Recent micropuncture studies indicate that the concentration of albumin in Bowman's space in the normal rat varies between 1 and 3 mg/dl [45, 46]. From these values it can be calculated that the glomerular sieving coefficient is less than 0.001, a value considerably smaller than that for anionic dextran sulfate with an identical Einstein-Stokes radius. A possible explanation for this size- and charge-independent enhancement of transglomerular dextran transport relates to its configuration. It has been suggested that dextran molecules, in contrast to globular proteins, are deformable and elastically compliant under the shear stresses imposed during convective flow through the glomerular membrane [48, 49]. Thus, these molecules may be deformed to elongated shapes and thus pass through pores far more easily than their unperturbed dimensions in solution might suggest.

Our finding of restricted transglomerular dextran transport in the presence of heavy proteinuria in diabetic nephropathy is similar to that already described in several other glomerular injuries, including MCN and glomerulonephritis in man [23, 50], and aminonucleoside nephropathy and nephrotoxic serum nephritis in the rat [24, 25]. The glomerular polyanion thought to be responsible for normal electrostatic retardation of anionic macromolecules is a sialic acid containing glycoprotein, which has been found to be depleted in both MCN [51] and in diabetic nephropathy [52, 53]. Loss of the electrostatic barrier may therefore contribute to the proteinuria in our patients with these two disorders.

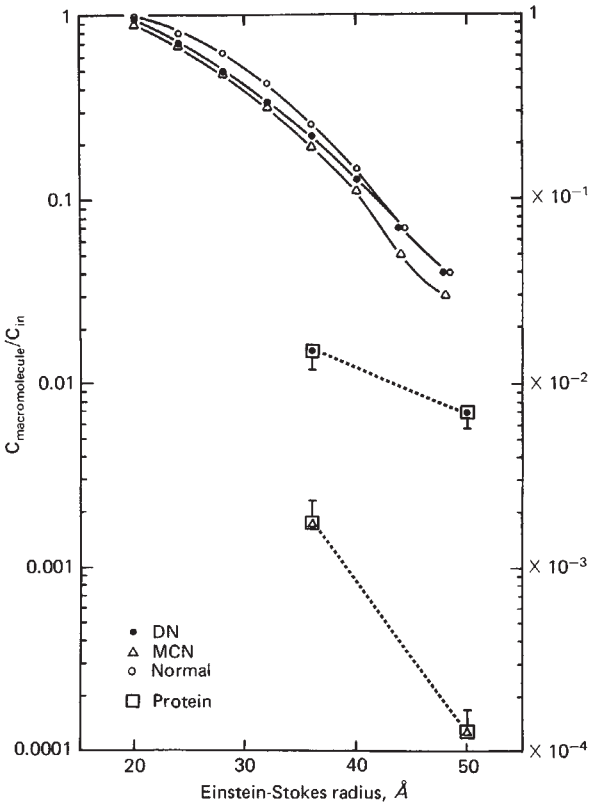

Fig. 2. *Fractional macromolecule clearance profiles in diabetic nephropathy* (• = dextran, ⊡ = protein) *and minimal change nephropathy* (Δ = dextran, ⊡ = protein). Also shown is the fractional dextran clearance profile in normal subjects (○).

The characteristics of the glomerular barrier in diabetic nephropathy differ from those in MCN, however, in two respects. Whereas the depression of θ_D in the interval 20 to 36 Å relative to normal persons was similar, θ_D for r > 36 Å was elevated in diabetic nephropathy relative to MCN (Fig. 2). In addition to increasing θ_d for larger dextran molecules, diabetic nephropathy was associated with

Table 2. Fractional dextran clearances in patients with diabetic nephropathy and in controls[a]

Groups	20 Å	24 Å	28 Å	32 Å	36 Å	40 Å	44 Å	48 Å
Diabetic nephropathy	0.99 ±0.04	0.72 ±0.04	0.52 ±0.04	0.36 ±0.03	0.23 ±0.02	0.14 ±0.01	0.08 ±0.01	0.04 ±0.01
Control groups								
(1) Normal volunteers	0.97 ±0.05	0.80[b] ±0.03	0.62[b] ±0.03	0.44[b] ±0.02	0.26[b] ±0.01	0.15 ±0.01	0.07 ±0.01	0.04 ±0.01
(2) Chronic renal disease	0.98 ±0.03	0.85[b] ±0.03	0.64 ±0.04	0.48[b] ±0.04	0.33[b] ±0.03	0.21[b] ±0.02	0.13[b] ±0.02	0.07[b] ±0.02
(3) Minimal change nephropathy	0.89 ±0.07	0.68 ±0.06	0.51 ±0.05	0.32 ±0.03	0.19 ±0.02	0.11[b] ±0.01	0.05[b] ±0.01	0.03 ±0.01

[a] Values are the means ± SEM.
[b] $P < 0.05$, compared with the diabetic nephropathy group.

progressive loss of discrimination between the test proteins and dextran molecules of equivalent size. Fractional clearance of dextran (r = 36 Å) was 110 times that for albumin (r = 36Å), whereas fractional clearance of dextran (r = 48 Å) was 230 times that of IgG (r = 50 Å) in minimal change nephropathy. In contrast, these differences were much less in diabetic nephropathy, being 18- and 7-fold, respectively (Fig. 2). Although a loss of the charge selective properties of the glomerular capillary wall might contribute to the proteinuria of both MCN and DN, there also appears to be a loss of shape discrimination in diabetic nephropathy.

The presence of a few isolated membrane defects permitting the unrestricted passage of large plasma proteins, combined with a shift in distribution of pores to those of smaller size, might account for our findings in diabetic nephropathy. A shift to smaller pore size would explain the depression of fractional dextran clearances ($r < 36$ Å) but not the normal fractional clearances of larger dextran molecules ($r = 40$ to 48 Å). Ideally, if this hypothesis of a few scattered large membrane defects superimposed on a shift to pores of smaller size were true, the fractional clearance for dextrans with a $r > 50$ Å would be predicted to decline to a finite and constant value between 0 and 0.04. In contrast, θ_D for progressively large molecules above 50 Å in normal controls would be predicted to decline asymptotically towards zero. Given the low concentration of appropriately large dextran molecules in the injectate and the analytical error involved in the determination of urinary dextran concentration at such low clearance rates, we have not been able to test this hypothesis, which must, therefore, remain speculative. It is noteworthy, however, that a focal foot-process degeneration with detachment of epithelial cells and denudation of the underlying GBM has recently been described in advanced diabetic nephropathy [54]. This process may totally disrupt the glomerular barrier to filtration of proteins and hence provide a structural basis for large membrane defects that do not discriminate among size, charge, or shape of macromolecules. Further investigation of human or experimental diabetic nephropathy, combining a structural approach with fractional clearances of larger test macromolecules, will be required to explore this hypothesis and to define more precisely the mechanism of proteinuria in this disorder.

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