Structural-Functional Relationships in Diabetic Nephropathy

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Abstract. Renal biopsies in 45 patients with insulin-dependent diabetes mellitus (IDDM) were examined by semiquantitative light microscopy and quantitative electron microscopic stereologic morphometry. In these 14 males and 31 females, aged 13–52 yr, who had had IDDM for 2.5–29 yr there was no strong relationship between either glomerular basement membrane (GBM) thickness or mesangial expansion and duration of IDDM. There was only a weak relationship between the thickness of the GBM and expansion of the mesangium. Thus, GBM thickening and mesangial expansion in IDDM occur at rates that often differ from one another and that vary greatly among patients. The clinical manifestations of diabetic nephropathy, albuminuria, hypertension, and decreased glomerular filtration rate related poorly or not at all to GBM thickening. In contrast, all light and electron microscopic measures of mesangial expansion were strongly related to the clinical manifestations of diabetic nephropathy, although in the absence of these clinical findings, it was not possible to predict the severity of any of the diabetic glomerular lesions. Mesangial expansion had strong inverse correlations with capillary filtering surface area density. It is hypothesized that mesangial expansion could lead to glomerular functional deterioration in IDDM by restricting the glomerular capillary vasculature and its filtering surface. However, capillary closure, glomerular sclerosis, and interstitial fibrosis could also contribute to the clinical manifestations of this disorder.

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Introduction

Renal failure ultimately develops in ~40% of patients with insulin dependent diabetes mellitus (IDDM)[1] (1, 2), and diabetes represents the single most common disease leading to renal insufficiency in adults (3). Renal failure in diabetes ultimately results from lesions that develop over many years. The natural history of nephropathy in IDDM is characterized by a prolonged period of clinical silence during which subtle functional abnormalities, including increased glomerular filtration rate (GFR) (4), and exercise-induced (4) or resting microalbuminuria (5), as well as increased renal size (4), can usually be documented. This is followed after an average of 15–20 yr by overt proteinuria, hypertension, and an inexorable decline in GFR (2, 6, 7). Qualitative descriptions of the lesions of diabetes have been recorded by many authors and recently reviewed elsewhere (8). Quantitative studies of structural-functional relationships in human diabetic nephropathy are rare. However, in 1959 Gellman et al. (9) reported their observations in 53 patients with diabetes of 8 to 24 yr duration and an age of onset of 5 to 70 yr. Despite this obviously mixed group of diabetic patients and the use of only semiquantitative light microscopic observations, these workers were able to correlate hypertension, proteinuria, and renal failure with the severity of diffuse glomerulosclerosis but were unable to define the importance of other structural changes such as glomerular basement membrane (GBM) thickening or to explain how diffuse glomerulosclerosis caused renal failure. More recently Gundersen and Østerby, examining renal tissues of six patients, five with renal insufficiency, indicated a relationship between the percentage of occluded glomeruli and levels of serum creatinine (10). They argued that enlarged open glomeruli represented a mechanism to compensate for functional loss of closed glo-

1. *Abbreviations used in this paper:* EM, electron microscope; GBM, glomerular basement membrane; GFR, glomerular filtration rate; IDDM, insulin-dependent diabetes mellitus; IME, index of mesangial expansion; NS, not significant; PAS, periodic-acid Schiff; S/V, relative surface densities of the peripheral capillary wall and of the endothelial-mesangial interface; UAE, urinary albumin excretion rate.

meruli and that these open glomeruli did not manifest marked "deposition of basement membrane material." Bader et al. (11) suggested that cortical interstitial fibrosis played the major role in the development of renal insufficiency. Since these studies presented somewhat contradictory views, we undertook a further, more detailed examination of structural-functional relationships in diabetic nephropathy.

Methods

Patients

These studies included 45 patients, 14 males and 31 females, aged 13–52 yr (29.7±9.3 yr, mean±SD) who had had IDDM for 2.5–29 yr (18.5±6.2 yr) and whose age of onset was from 9 mo to 30 yr (11.2±7.3 yr). The duration of IDDM since puberty ranged between 2.5 and 27 yr (13.6±7.1 yr). 41 patients had renal biopsies performed as part of their evaluation as potential pancreas transplant recipients. Four patients had renal biopsies because of minimal proteinuria or hypertension developing between 5 and 11.5 yr after the onset of IDDM (two patients) or as baseline biopsies before the institution of strict glycemic control (two patients). 12 patients had either no detectable complications or minimal background retinopathy and/or changes in nerve conduction. 16 patients had moderate to severe retinopathy with no clinical nephropathy. One patient had only severe neuropathy. 16 patients had clinical nephropathy, usually with retinopathy and/or neuropathy. Thus, this group of patients may have a higher incidence of serious complications than the general population of IDDM patients (1). Informed consent for the studies performed was obtained from all patients or their parents. All potential pancreas transplant recipients were studied according to a protocol approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota. These patients were studied in the Clinical Research Center at the University of Minnesota Hospitals.

Renal studies

Functional studies. Creatinine clearances were determined by standard laboratory procedures. At least two, and usually three, clearance studies were performed on each patient before renal biopsy, and the means of these clearances, where necessary corrected up to 1.73 M^2, were calculated. The normal values for our laboratory are 95–115 ml/min per 1.73 M^2. 24-h urinary albumin excretion rates (UAEs) were measured by nephelometry using the Beckman kit (Beckman Instruments Inc., Fullerton, CA) (12). Blood pressure data were considered valid only when repeated determinations (at least 10) were available over at least several days. Criteria of the Joint National Committee on the Detection, Evaluation and Treatment of High Blood pressure (13) and the Task Force on Blood Pressure Control in Children (14) were used in defining hypertension. Since several patients defined as hypertensive were receiving antihypertensive drugs, only the presence or absence of hypertension, and not the magnitude of hypertension, was considered.

Morphologic studies. The 45 patients had 47 renal biopsies. Five patients had needle biopsies at the time of surgery for pancreas transplantation. All others were percutaneous renal biopsies accomplished with only one complication, the inadvertent biopsy of a spleen. This resulted in no serious sequelae. Most of the biopsy specimens were immediately examined under a dissecting microscope to ensure adequacy of sample based upon the number of glomeruli seen on the surface of the renal core and to ensure correct division of the core, performed under the dissecting microscope, to provide glomeruli for light and electron microscopy.

A part of the renal biopsy specimen was placed in Zenker's fixative for 1 h, washed in tap water, and processed in paraffin by usual techniques. The sections were cut in sequence, stained with periodic-acid Schiff (PAS), and numbered so that cuts through the specimen could be selected that presented two or three separate populations of glomeruli for examination. The number of glomeruli per specimen was 26±10 (mean±SD). Kidney tissues (1-mm cubes) were also fixed for at least 4 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, 290 mosm/liter (15). Postfixation for 1 h in 1% osmium in 0.1 M cacodylate buffer was followed by embedding in Epon 812 (Polysciences, Inc., Warrington, PA). Thick sections (1 μm) were cut and stained with toluidine blue in order to randomly select the centermost, intact glomerulus in the block for electron microscopy (15). Ultrathin sections (50–70 nm) were obtained on a microtome (MT-2B; E. I. Du Pont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT), placed on Forvar-coated 50-mesh grids, and stained with saturated uranyl acetate and lead citrate. A calibration grid (28,800 lines/inch; Ernest F. Fullam, Inc., Schenectady, NY) was photographed along with the micrographs of each glomerulus on an electron microscope (100CX; JOEL, Tokyo). After the centermost glomerulus in a block was randomly entered, 10–20 evenly spaced electron micrographs were obtained at 18,000 magnification, representing a total area of ~1,250–2,500 μm^2 (15). Sclerotic glomeruli were not studied.

Measurement and grading of morphologic parameters were done by light and electron microscopy as follows.

Light microscopy

All tissues were coded and blindly evaluated for the following parameters.

The index of mesangial expansion (IME) was determined by a semiquantitative estimate of the width of mesangial zones in each glomerulus (16); 0 was used as normal, 1.0 as twice normal thickness, 2.0 as three times normal thickness, etc. (Fig. 1, *A* and *C*). Half grades were assigned where appropriate. The mean of the grades for each glomerulus for IME was determined for each patient and represents the IME score reported here. 20 of the biopsies with IME evenly distributed from values of 0 to 4.0 were read blindly a second time. The mean difference between the two readings was 8%, with a mean difference in IME score of 0.13 (range 0–0.4). Thus, a 0.5 gradation in IME score represents a reproducible, discernible difference. This light microscopic parameter of IME correlated highly with electron microscopic measures of the mesangium (see below), including percentage total mesangium ($r = +0.86$, $P < 0.0005$), percentage mesangial matrix ($r = +0.89$, $P < 0.0005$), and percentage cellular mesangium ($r = +0.71$, $P < 0.0005$).

The index of interstitial fibrosis was determined as a semiquantitative estimate of the space occupied by fibrous tissue separating cortical tubules; 0 was used as normal, 1.0 as twice normal thickness, 2.0 as three times normal thickness, etc., in each 500× cortical field. Half grades were assigned where appropriate for each field and mean values were obtained for each patient. By careful examination of serial sections an effort was made to avoid inclusion of the interstitial fibrosis associated with glomerular sclerosis in this measure. These foci of interstitial fibrosis associated with tubular atrophy, marked tubular basement membrane thickening, and interstitial inflammation were not evaluated. It should be pointed out that normally there is little cortical interstitial fibrous tissue. Thus, an index of 2 or 3 represents

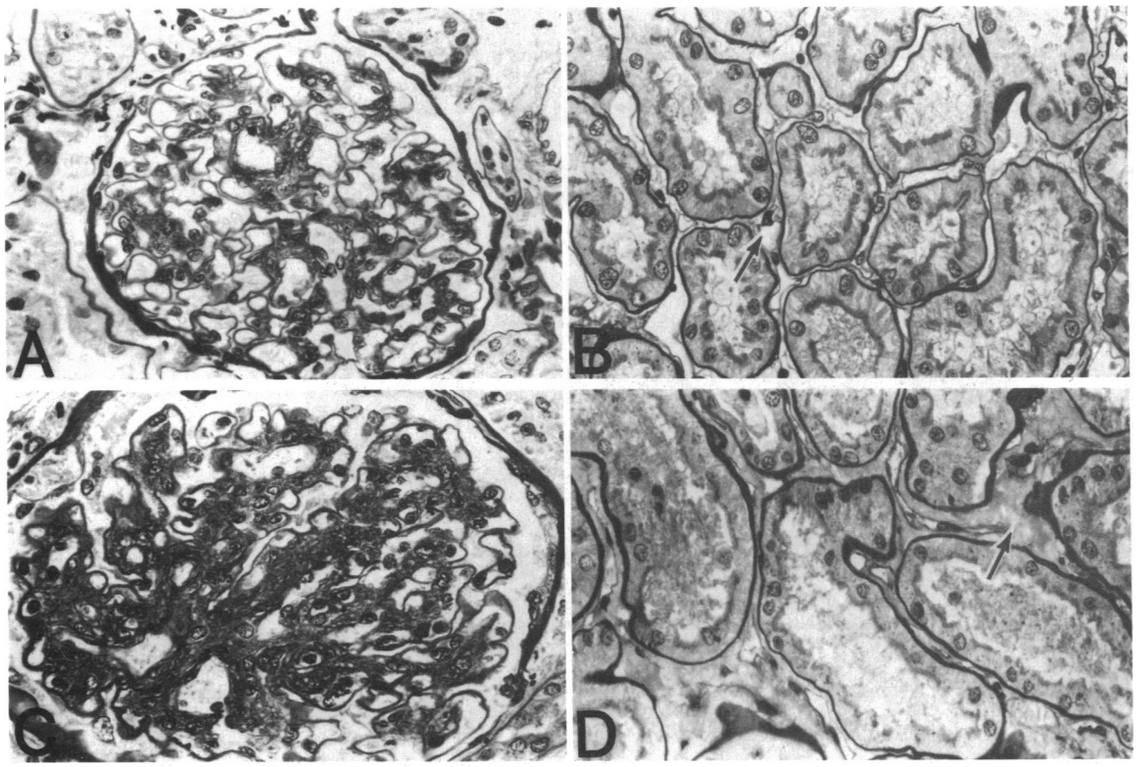

Figure 1. (*A*) Representative glomerulus from a biopsy with a mean IME of 1.25 (PAS × 350). (*B*) Representative cortical area with a mean index of interstitial fibrosis of 0.25 (arrow) in same biopsy as *A* (PAS × 350). (*C*) Representative glomerulus from a biopsy with a mean IME of 3.5 (PAS × 350). (*D*) Representative cortical area with a mean index of interstitial fibrosis of 2.75 (arrow) in same biopsy as *C* (PAS × 350).

a subtle finding easily overlooked unless specific examination for this parameter is carried out (Fig. 1, *B* and *D*).

The index of arteriolar hyalinosis was determined as a semiquantitative estimate of replacement of arteriolar smooth muscle by waxy, homogeneous PAS-positive material. Incomplete replacement of a few vessels was graded as 0.5, incomplete replacement of most vessels was graded as 1.5, complete replacement of approximately one-half the arterioles was graded as 2.5, and complete replacement of most of the arterioles was graded as 3.5.

Glomerular volumes were determined by the method of Gundersen and co-workers (17). In order to have sufficient glomerular profiles to provide valid measurement of this parameter (17), the patients were divided into three groups. The first group had relative areas of the total mesangium in the normal range for our laboratory (26% or less, see below). The second group had percentage total mesangium in the range of 27 to 36%. The third group had percentage total mesangium of 37% or more, values regularly associated with clinical diabetic nephropathy (see Results). Mean creatinine clearances for these three groups were 132±26, 108±9, and 73±27 ml/min per 1.73 M^2, respectively. These means are significantly different from one another ($P < 0.005$). Glomerular volumes representative of each of these three groups were calculated from a study of 502, 253, and 391 glomeruli per group, respectively. Since discrete glomerular volumes were not measured for each patient because many did not have adequate numbers of glomeruli in the biopsy specimens, estimates of absolute volumes of glomerular components and areas of glomerular surfaces in the three patient groups were not compared statistically.

Electron microscopy

GBM thickness was obtained by the orthogonal intercept method described by Jensen et al. (18) and previously used by us (19). In brief, a grid with eight evenly spaced intersecting lines (four horizontal and four vertical) is placed over each photomicrograph and GBM measurements are made at each point that a line on the grid intercepts an endothelial-GBM interface. The width is measured on a line orthogonal to the edge of the GBM at the endothelial side of the intercept (Fig. 2). 104±41 (mean±SD) measurements of GBM width were done on each biopsy. In normal subjects (based upon 118 individuals aged 9 to 65 yr) the GBM width was 349±49 nm (20). This mean value does not take into account minor but highly statistically significant variations in normal GBM width with age and sex (20), subtleties that are beyond the scope of the present work.

Fractional volumes of glomerular components were measured by placement of the eight line grid having 16 intersections representing 16 coarse points and an additional 64 fine points (+, Fig. 2) over each

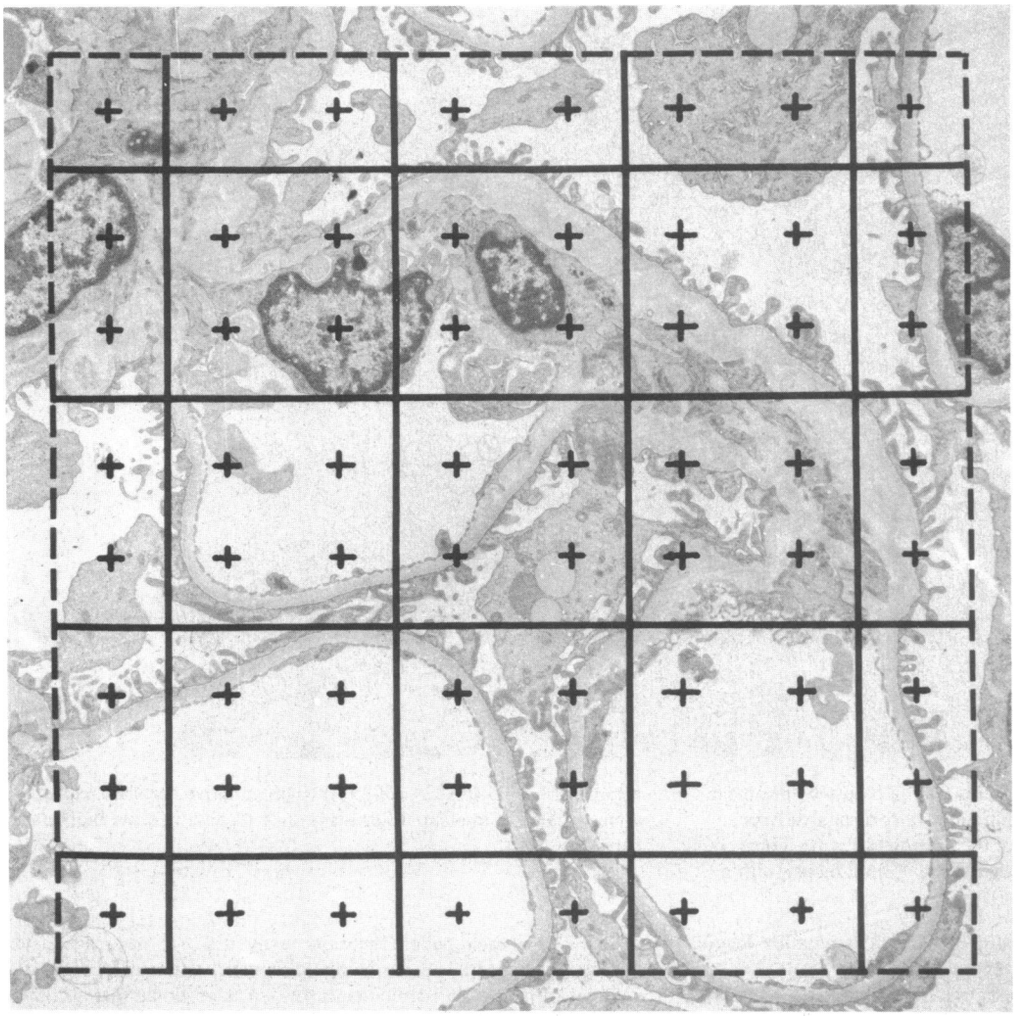

Figure 2. Electron photomicrograph with superimposed grid illustrating lines (solid) used to determine points of intersection for measuring GBM thickness and intersections with surfaces used to estimate surfaces. Points for determining fractional volumes of glomerular structural components include the 16 line intersections (coarse points) as well as the four fine points around each coarse point (+).

electron micrograph to determine the proportion (or fractional volume) of points falling on the cellular and matrix components of the glomerulus (15). A total of 3,312±1,448 points on 2–6 (3.4±1.3) glomeruli were evaluated per biopsy.

The transition between the peripheral capillary area and the mesangium was determined, as previously described (15), on the basis of widening of the distance and disappearance of the parallelism between the endothelial and epithelial cells (15). This demarcation was used to identify the beginning of the mesangium as well as the end of the peripheral capillary wall (i.e., the endothelial cell-GBM-epithelial cell interface). The fractional volumes of the total mesangium and its matrix and cellular components were measured, excluding the glomerular basement membrane lying between the epithelial cell and the mesangium (Fig. 3). The average area of the total mesangium in 118 normal subjects was 14.2±4.1%, with the matrix and cellular components each averaging 7.1±2.4% (20). Within the age range studied these mesangial measures were neither age nor sex dependent (20).

Relative surface densities (S/V) of the peripheral capillary wall and of the endothelial-mesangial interface (Fig. 3) were measured with the grid by standard stereologic techniques (15). S/V represents twice the number of times the lines on the grid intersected the surface being studied divided by the total length of the lines. An average line length of 2,593±1,119 μm was examined per biopsy over an average reference area of 2,521±1,088 μm^2. The mean value in our laboratory for S/V of the peripheral capillary wall among the 118 normal subjects is 0.133±0.022.

Validation of morphometric analyses. To assess the validity of assigning a discrete value to each of the electron microscopic morphometric parameters, the following study was performed. Each of 11 patients, evaluated as described above, had an additional two to four glomeruli per biopsy photographed by electron microscopy so as to permit a total reconstruction of each glomerular cross-section by use of the photomontage technique previously described by Østerby (21). The magnification used was the same as in the random sampling

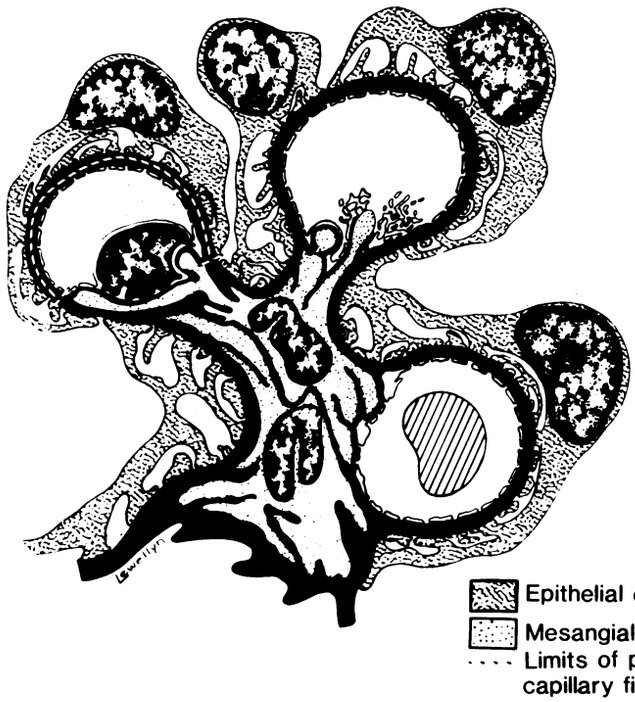

Figure 3. Schematic representation of glomerular tuft illustrating the limits of the peripheral capillary filtration surface (adapted from Vernier et al. [47]).

technique described above. A 30 × 30 fine point grid (total 900 points) with 15 horizontal and 15 vertical lines was used to measure percentage total mesangium and S/V of the peripheral capillary wall. Unlike the grid shown in Fig. 2, all of the points in this larger grid were equidistant from one another. The correlations of percentage total mesangium and S/V of the peripheral wall, when the montage and the random sampling techniques were compared, were +0.94 ($P < 0.001$) and +0.88 ($P < 0.001$), respectively, thus indicating that the random glomerular sampling method and the 80-point grid provide discrete and reproducible values for individual glomerular structural components. These studies confirm the impression given by light microscopy that interglomerular variability in the severity of diabetic glomerular lesions is not great and is unlikely to influence markedly the overall results of the morphometric analyses.

Statistical methods

Relationships between structural and functional parameters were examined by regression analyses (method of least squares) and chi-square calculations. In addition, where appropriate, multiple regression analyses were performed. Since the number of glomeruli in each biopsy sample (at least 10 per biopsy) could influence the accuracy, through sampling error, of the estimate of percentage sclerotic glomeruli, this value was treated by arcsine transformation and, along with confidence intervals reflecting sample size, was plotted on an arcsine scale (22). These transformed values for percentage sclerotic glomeruli were then correlated with other structural or functional variables by use of regressions and chi-square weighted for number of glomeruli per biopsy (23). Because numerous regression analyses were performed, only P values < 0.005 were considered significant.

Results

Duration. There was no significant relationship between GBM thickness and duration since detection of diabetes (Fig. 4). There was no significant correlation between the IME (Fig. 5) and duration of diabetes. Because of the close correlation of IME with the percentage total mesangium ($r = +0.86$, $P < 0.0005$), percentage matrix mesangium ($r = +0.89$, $P < 0.0005$), and percentage cellular mesangium ($r = +0.71$, $P < 0.0005$) as measured by electron microscope (EM) stereology, these ultrastructural parameters did not correlate significantly with duration of diabetes. Furthermore, duration of diabetes since puberty was predictive of neither the light or the EM measures of mesangium nor of GBM thickness. The tendency for an increasing range of IME values with 10 yr or more of diabetes (Fig. 5) suggests a marked variability in the rate of development of mesangial changes.

Relationship of GBM and mesangial expansion. There was a statistically significant but relatively weak relationship between GBM thickness and percentage total mesangium (Fig. 6).

Mesangial thickness and other glomerular structural parameters. The IME and the EM measures of the total mesangium and its individual components all had striking inverse relationships with the S/V of the peripheral capillary wall, as illustrated in Fig. 7. Thus, it appears that mesangial expansion in diabetic nephropathy occurs at the expense of the peripheral

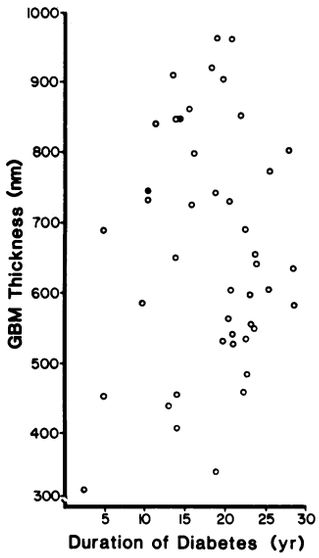

Figure 4. Relationship of GBM thickness and duration of diabetes. $r = +0.01$, NS. In this and all subsequent figures ○ represents patients with single biopsies and ● or ■ represents two sequential biopsies in the same patient.

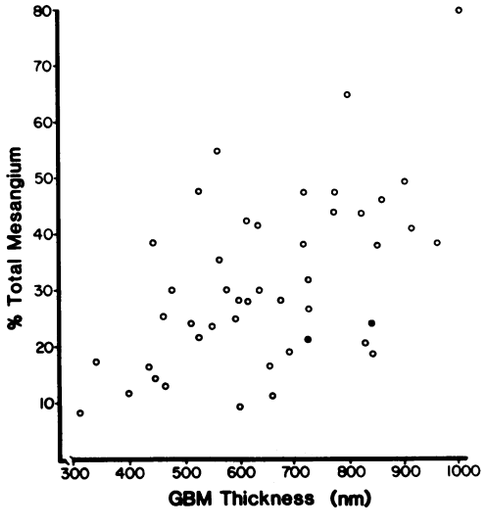

Figure 6. Relationship of GBM thickness and percentage total mesangium. $r = +0.56$, $P < 0.0005$.

capillary filtration surface. This is further reflected in an inverse relationship between the peripheral capillary surface and the endothelial-mesangial interface ($r = 0.60$, $P < 0.0005$).

Mesangial thickness and interstitial fibrosis. The index of interstitial fibrosis and IME correlated directly (Fig. 8). The index of interstitial fibrosis also correlated with the percentage total mesangium ($r = +0.80$, $P < 0.0005$) and its cellular and matrix components. Although the index of interstitial fibrosis tended to vary directly with GBM thickness, this relationship was not significant (NS) ($r = +0.47$).

Glomerular structural alterations and creatinine clearance. There was no significant relationship between creatinine clearance and GBM thickness ($r = -0.42$, NS) and index of arteriolar hyalinosis ($r = +0.18$, NS). There was a significant relationship between percentage sclerotic glomeruli (arcsine transformation) and creatinine clearance (Fig. 9). However, multiple regression analysis of percentage total mesangium or IME and percentage sclerotic glomeruli upon creatinine clearance demonstrated that creatinine clearance related significantly to these mesangial

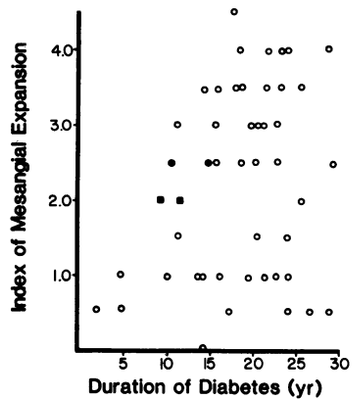

Figure 5. Relationship of the IME representing the mean glomerular score for each patient and duration of diabetes. $r = +0.33$, NS.

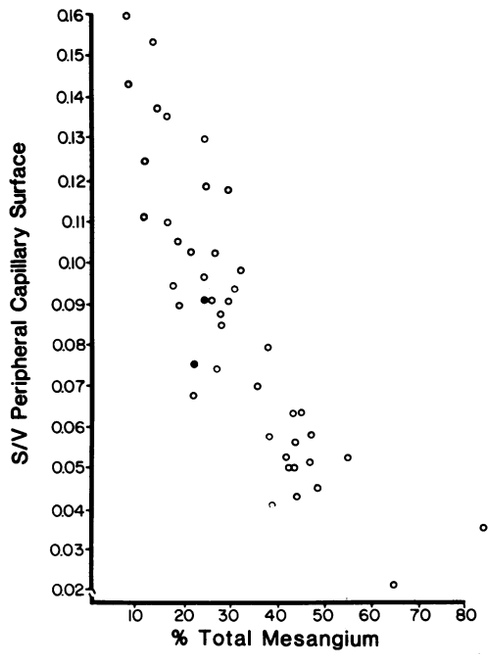

Figure 7. Relationship of percentage total mesangium and S/V of the peripheral capillary surface. $r = -0.86$, $P < 0.0005$.

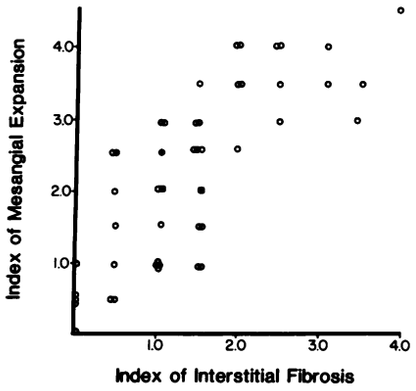

Figure 8. Relationship of IME (mean of scores for individual glomeruli in biopsy) and index of interstitial fibrosis (mean of scores of cortical fields in biopsy). $r = +0.80$, $P < 0.0005$.

indices ($P < 0.0005$) and not to percentage sclerotic glomeruli. In fact, all measures of mesangial expansion (as illustrated in Fig. 10) had the strongest inverse correlation with creatinine clearance, and the inclusion of percentage sclerotic glomeruli with IME in a multiple regression analysis did not improve the prediction of creatinine clearance ($r = +0.67$). Since S/V of the peripheral capillary surface was inversely correlated with total mesangial volume, it follows that direct correlations with this surface parameter and creatinine clearance would be observed ($r = +0.56$, $P < 0.0005$). Nonetheless, it is clear that there can be overlap in the magnitude of the disturbances in glomerular structure among patients with and without decreased creatinine clearance (Fig. 10 and Table I). As expected from the relationship of mesangial parameters and index of interstitial fibrosis (Fig. 8), the latter also correlated inversely with creatinine clearance ($r = -0.78$, $P < 0.0005$).

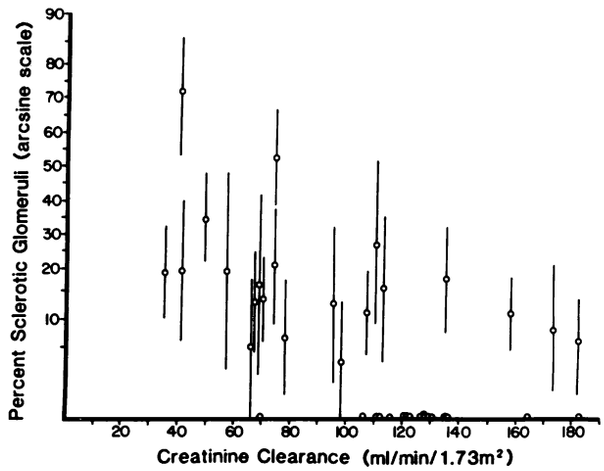

Figure 9. Relationship of percentage sclerotic glomeruli and creatinine clearance. Percentage sclerotic glomeruli plotted on the arcsine scale. The percentage sclerotic glomeruli and the estimated 95% binomial confidence limits (vertical lines) estimated from the number of glomeruli in the kidney sample are presented for each biopsy. $r = -0.58$, $P < 0.005$.

Renal structural alterations and 24 h urinary albumin excretion (UAE). No structural glomerular parameter precisely predicted UAE. However, all patients with total mesangial volume >37% (Fig. 11) and S/V of the peripheral capillary <0.065 (chi-square = 37, $P < 0.0005$) had >400 mg UAE, whereas all patients with less marked disturbances in these parameters had <200 mg UAE. However, it should be pointed out that mesangial expansion and its correlated change, S/V, of the peripheral capillary surface, could be approaching the range at which heavy albuminuria regularly occurs without being reflected in a substantial increase in UAE (Fig. 11 and Table I). Although there was a trend towards a relationship between GBM thickness and UAE, this did not reach statistical significance (Fig. 12). Thus, marked GBM thickening was compatible with minimal UAE whereas heavy albuminuria could be associated with lower increases in GBM thickness (Fig. 12). In addition, an index of interstitial fibrosis >2.0 was associated with >400 mg/24 h of urinary albumin (chi-square = 28, $P < 0.0005$). Identical chi-square analyses were obtained when UAE was expressed as micrograms albuminuria per milliliter glomerular filtrate. 12 of the 24 patients with normal creatinine clearance values and no hypertension had UAEs in the measurable range of the test used (Table I). In these 12 patients there were no significant correlations between UAE and any of the structural parameters listed (Table I).

Renal structural alterations and hypertension. Mesangial expansion to >37% of glomerular volume (Table II), and S/V of the peripheral capillary surface of <0.065 (chi-square = 33, $P < 0.0005$) were highly predictive of hypertension. Three patients with fractional mesangial areas of 44, 47, and 55% had creatinine clearance values of 113, 120, 128 ml/min per m^2, respectively, and UAEs of >400 mg per 24 h. These patients each had S/V of the peripheral capillary surface of <0.065. All other patients with mesangial expansion > 37% had reduced creatinine clearances. GBM thickness was not related to hypertension (Table III). The index of interstitial fibrosis graded as >2.0 predicted hypertension (chi-square = 22, $P < 0.0005$).

Estimates of absolute glomerular structural parameters. Discrete glomerular volume measurements were not obtained in most patients because the numbers of glomeruli in many of the percutaneous biopsies were insufficient for accurate determination of this parameter. In patients in the group with percentage total mesangium ≤ 26% (Table IV), glomerular volume was not increased whereas in patients with established mesangial expansion (27–37%) glomerular volume was distinctly increased. However, there was no further increase in glomerular

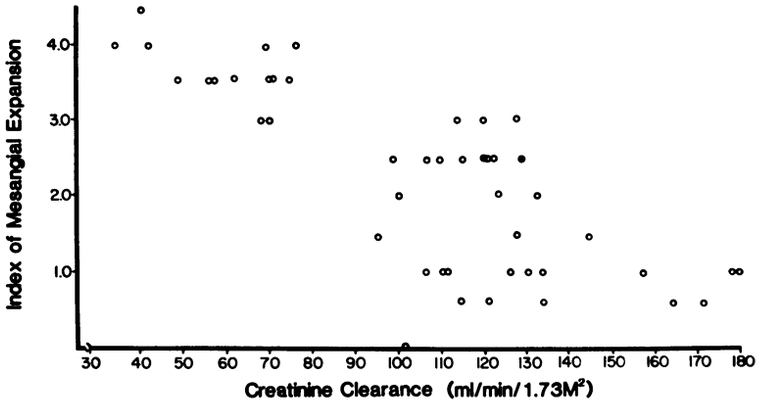

Figure 10. Relationship of index of mesangial expansion (mean of scores for individual glomeruli in biopsy) and creatinine clearance. $r = -0.72$, $P < 0.0005$.

volume in patients with marked mesangial expansion (>37%). As expected from the method of patient allocation into groups, absolute mesangial volume increased markedly as fractional mesangial volume increased. The absolute peripheral capillary filtering surface area was not increased in patients with total mesangium ≤ 26% but, interestingly, was increased in the 27–37% group (Table IV). Absolute peripheral capillary filtering surface was decreased in patients with marked mesangial expansion (Table IV). The absolute endothelial-mesangial surface area was increased in diabetic patients with moderate mesangial expansion, but no further increase was noted in those with more severe mesangial expansion (Table IV).

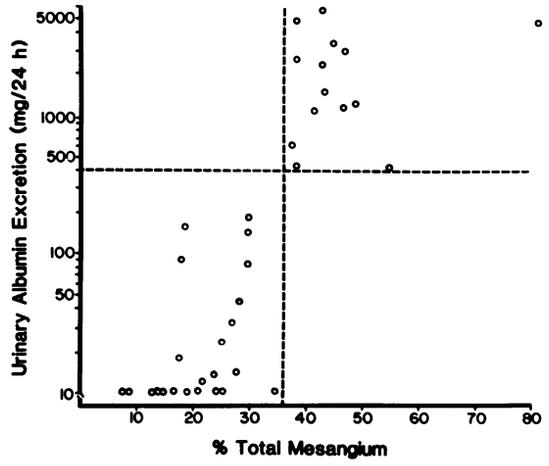

Figure 11. UAE and percentage total mesangium. The interrupted lines represent the graphic limits of the chi-square, which was calculated to be 27, $P < 0.0005$. In this and next figure note the triple log scale for UAE; in both figures points near the abscissa (at 10 mg/24 h) represent UAE rates below the sensitivity range of the urinary albumin test.

Discussion

Most of the patients studied here had renal biopsies performed because they manifested one or more of the major complications of IDDM. Thus, they may not be cross-sectionally representative of the spectrum of IDDM patients since the incidence of manifest complications may be somewhat higher in our patients (1, 2). Nonetheless, we have no reason to question the validity of the renal structural-functional relationships found. The structural changes described were determined for individual patients by the use of both quantitative EM stereologic and semiquantitative light microscopic techniques. Measurements of GBM thickness involved sampling techniques that have been shown to have a small coefficient of variation and that provide biologically meaningful results (18, 24). In order to

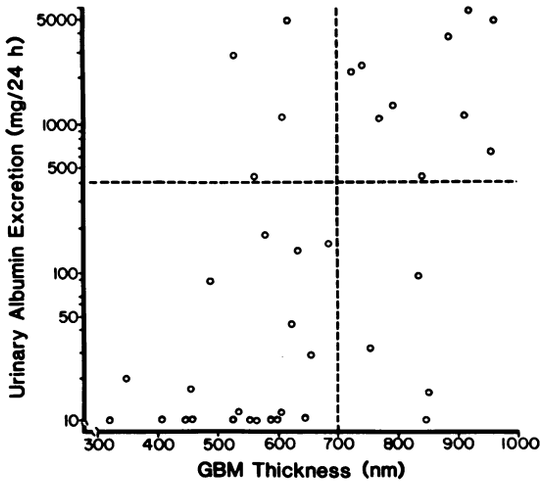

Figure 12. UAE and GBM thickness. The interrupted lines represent the graphic limits of the chi-square, which was calculated to be 5.98, NS.

Table I. Morphologic Findings and UAE in Patients Without Clinical Diabetic Nephropathy

Patient No.	Duration	IME*	IIT‡	IAH§	% Hyalinized glomeruli	% Total mesangium	% Matrix mesangium	% Cellular mesangium	S/V	GBM	UAE
	yr									*nm*	*mg/24 h*
2	5	1.5	0.25	.5	0	27	16	11	0.103	692	ND‖
6	15	2.5	1.0	2.0	0	24	17	7	0.091	850	13.6
7	12	1.5	1.0	2.0	0	21	14	7	0.074	843	NM¶
9	23	2.5	1.0	2.0	24	30	20	10	0.091	483	87.0
12	29	2.5	2.0	2.0	16.6	30	20	10	0.119	732	192.3
13	21	1.5	0.5	1.5	0	36	24	12	0.076	563	NM
14	17	0.5	0.0	0.25	0	14	7	7	0.135	475	NM
18	2.5	0.5	0.5	0.25	0	7.5	2.4	5.1	0.160	610	NM
20	22	2.0	0.5	1.0	0	24.5	15.2	9.3	0.072	533	12.5
21	26	2.0	1.0	2.0	0	28	20	7	0.087	604	14.2
26	24	1.0	1.0	1.5	0	12	7	5	0.124	657	19.2
27	29	1.5	1.5	2.0	0	28	19	9	0.086	624	46.8
31	10	1.0	0.5	0.75	0	9	5	4	0.144	593	NM
32	14	0.0	0.0	0	5	13	7	6	0.111	397	NM
33	14	1.0	0.0	2.5	12.5	18	11	7	0.089	837	92.3
35	27	0.5	0.5	2.0	0	15	8	7	0.137	441	NM
37	16	1.0	0.5	1.0	0	24	16	8	0.093	521	NM
39	14	1.5	1.5	2.0	0	17	12	5	0.110	651	NM
40	23	1.5	1.5	.75	10	19	12	7	0.106	693	160
43	5	0.5	0.0	.25	0	13	6	7	0.153	455	NM
44	24	1.0	0.25	0	0	24	14	10	0.097	551	NM
46	19	1.0	1.25	0.5	0	17	10	7	0.136	337	20
47	22	1.5	1.5	2.0	0	26	16	10	0.113	464	12.7
48	18	2.5	1.5	2.0	0	27	19	8	0.074	728	34

* IME, index of mesangial expansion. ‡ IIT, index of interstitial thickness. § IAH, index of arteriolar hyalinosis. ‖ ND, not done. ¶ NM, not measurable.

Table II. Relationship of Blood Pressure and Total Mesangial Volume

	Hypertensive	Normotensive
% Mesangial volume > 37	15	0
% Mesangial volume < 37	1	24

Chi-square = 36, $P < 0.0005$.

determine the validity of ascribing discrete values to each patient for stereologic measurements that have potential for greater interglomerular and interindividual variation (25), we compared our stereologic techniques for mesangial and surface measurements with a more thorough sampling method (21) in patients from whom additional glomeruli were available for study. We found a very high correlation for measures of relative mesangial volume and peripheral capillary surface area in comparing these two morphometric approaches.

The strong direct correlations between the light microscopic IME and the EM parameters of percentage total mesangium, percentage mesangial matrix, and percentage mesangial cells indicate that the semiquantitative light microscopic IME based on impressions of an average of 25 glomeruli per biopsy is a useful tool and should not be dismissed as merely subjective. Thus, these findings strengthen the light microscope studies of Gellman et al. (9). Conversely, these results support the thesis that mesangial EM measurements, based upon a relatively small glomerular sample and perhaps subject to sectioning vagaries (25), accurately represents the general glomerular population.

The failure to find close relationships between the duration of Type I diabetes and renal lesions is not surprising given the known variability in the clinical expression of diabetic nephropathy among IDDM patients (2, 6, 26, 27). However, a study of a more representative patient population could provide a clearer view. Perhaps of equal interest are the relatively weak relationships between GBM thickness and measures of the mesangium. That these may be independent variables in the evolution of the glomerular lesions of diabetes is further suggested by studies of rats cured of diabetes with pancreas transplantation which demonstrated reversibility of increased mesangial matrix material and irreversibility of GBM thickening (15, 16, 19). These and other studies (28) are consistent with there being different production and turnover rates for GBM and mesangial matrix constituents. Furthermore, unilateral nephrectomy accelerates mesangial expansion but not GBM thickening, suggesting different pathogenic mechanisms (29). The biochemical makeups of GBM and mesangial matrix appear to be remarkably complex and in many ways different from one another (30). Immunohistochemical changes in GBM and mesangial matrix constituents do not completely parallel one another in diabetic nephropathy (31). It has been established that all of the recognized antigens normally present in the developed adult mesangium are increased in the nonsclerosed glomeruli of diabetic patients with mesangial expansion. In addition, certain antigens present in normal human fetal glomeruli but absent from normal adult mesangial regions appear in widened mesangial areas in Type I diabetic patients. Thus, the increase in the volume of the mesangium in diabetes is based on a complex polyantigenic expansion of glycoproteins, collagens, and as yet unidentified structural components. Whether this represents increased synthesis, decreased breakdown, or both, of these components is unknown. Previous studies (32) have demonstrated impaired macromolecule removal rates in expanded mesangial areas in rats with long-standing diabetes; however, further studies will be required to resolve this issue, which may be central to the understanding of the pathogenesis of diabetic nephropathy.

Table III. Relationship of Blood Pressure and GBM Thickness

	Hypertensive	Normotensive
GBM > 700 nm	9	6
GBM < 700 nm	7	19

Chi-square = 2.6, NS.

Our studies demonstrate that clinical diabetic nephropathy as defined by Albustix (Ames Chemical Co., Inc., Farmington, MI) positive proteinuria, hypertension, and declining GFR (33) does not become manifest until renal lesions become far advanced. In fact, our patients with the earliest findings of clinical nephropathy uniformly had severe glomerular lesions. These clinical abnormalities were closely related to measures of the mesangium and peripheral capillary surface, whereas GBM thickening per se appears to be a poor candidate for explaining altered permeability to protein hypertension and renal insufficiency in diabetic nephropathy.

It is hypothesized that progressive mesangial expansion could ultimately contribute to the clinical manifestations of diabetic nephropathy by influencing contiguous structures. In the present studies we demonstrated an inverse relationship between relative mesangial volume and peripheral capillary filtering surface density, and an inverse relationship between this filtering surface density and that of the endothelial-mesangial interface. The surface of the glomerular capillary wall responsible for filtration has yet to be precisely defined. However, tracer studies suggest that the peripheral capillary surface is more important in filtration than is the endothelial-mesangial interface (34). That the peripheral capillary surface is an important determinant of GFR is suggested by studies early in diabetes in man defining a correlation between the increase in this surface and the increased GFR, (35) whereas the power of the correlation of GFR and filtration surface in our studies derives from patients with reduced GFR.

Expression of glomerular stereologic parameters as absolute

Table IV. Glomerular Morphometric Parameters Expressed as Absolute Values in Diabetic Patients and Normal Subjects

	Normal subjects	Diabetic patients with total mesangium ≤26%	Diabetic patients with total mesangium 27–37%	Diabetic patients with total mesangium >37%
Number of subjects	26	19	12	14
Glomerular volume ($\mu m^3 \times 10^6$)	1.3	1.4	2.4	2.4
Total mesangial volume/glomerulus (μm^3)	0.19	0.26	0.7	1.2
Peripheral capillary filtering surface/ glomerulus (μm^2)	0.17	0.16	0.23	0.12
Endothelial surface mesangial-interface/glomerulus (μm^2)	0.06	0.08	0.16	0.17

values derived from estimates of glomerular volume must be considered to be preliminary since a discrete glomerular volume measurement for each patient was not performed because sufficient numbers of glomeruli for accurate determination of this parameter (17) were not available in many of the percutaneous biopsies. For this reason patients were grouped for glomerular volume studies, and statistical comparisons among these groups were not performed. Nonetheless, it appears from these studies that glomerular volume is not increased in diabetic patients with little or no mesangial expansion compared with normal subjects. This finding is similar to the results of individual glomerular volume measurements in a small group of patients studied 1 to 6 yr after onset of IDDM (36). It appears from these studies that glomerular volume, increased in patients with mild to moderate mesangial expansion, does not further increase in patients with severe mesangial thickening. It is clear that, by design, absolute mesangial volume is greatest in the latter group. Note that peripheral capillary surface area in patients with mild-to-moderate mesangial expansion seems to be greater than in normal subjects or in diabetics with relative mesangial areas that are within the normal range. Although the mean GFR in this group of patients is in the normal range, it is significantly lower than the mean for patients with mesangial volume within the normal range, the latter demonstrating the phenomenon of hyperfiltration so often seen in IDDM (4). If one accepts the hypothesis that the peripheral capillary wall of the glomerulus expands to maintain its filtering capacity (37), then the increase in the peripheral capillary filtering surface and the relative decrease in GFR in patients with mild-to-moderate mesangial expansion could reflect a decrease in the intrinsic hydraulic permeability of the glomerular capillary wall to the passage of small molecules (38, 39). It is thus reasonable to suggest that patients with advanced mesangial expansion could have both decreased absolute filtering surface and decreased permeability to small molecules as the explanation for their observed decrease in GFR. Individual absolute structural measures along with more precise functional studies are required to adequately defend this hypothesis.

Although glomerular sclerosis cannot, by itself, explain decreased GFR in diabetic patients with mild to moderate renal insufficiency, this could be an important factor in some patients. Furthermore, capillary closure (40) within nonsclerotic glomeruli cannot be excluded as contributing to diabetic renal failure. In fact, this process could explain the decreased peripheral capillary filtering surface without further increase in the estimate of the absolute surface of the mesangial-endothelial interface in the patients with more advanced mesangial expansion (Table IV).

The present studies indicate that GBM thickening per se is unlikely to be responsible for the development of overt proteinuria in diabetic nephropathy. In contrast, measures of the extent of mesangial expansion and peripheral capillary surface were related to levels of albuminuria that would be clinically detectable by Albustix. It is known that the microalbuminuria of diabetic patients without clinical nephropathy (4, 5) is reversible by the institution of short-term strict glycemic control (5), whereas in diabetic patients with overt proteinuria, fractional IgG and albumin clearances continue to increase and GFR continues to decrease despite 6 mo of strict glycemic control (41). It is also known that the onset of overt proteinuria ushers in the development of a progressive deterioration in the macromolecular permselective characteristics of the glomerular barrier (38, 42). It is, therefore, reasonable to consider that microalbuminuria and overt clinical proteinuria in diabetes reflect different pathogenetic processes. Myers and co-workers (38, 42) have described a loss of size-selective and charge-selective properties of the glomerular barrier in advanced diabetic nephropathy in man, which is similar to the pattern of altered glomerular permeability to protein seen in rats subjected to subtotal nephrectomy (43). If this analogy is meaningful, it may be that advanced diabetic nephropathy, perhaps due to the associated diminution in available glomerular capillary filtering surface, results in compensating alterations in intraglomerular hemodynamics that mimic the physiology of the remnant kidney. If these hypotheses are correct, they will be consonant with the concept that once marked glomerular lesions have developed, intraglomerular

physical forces result which, independent of the diabetic state, produce progressive glomerular injury. Glomerular sclerosis would aggravate this situation but would not be a necessary component. These thoughts are consistent with the observation that treatment of hypertension retards the decline in GFR and reduces proteinuria (44) whereas precise glycemic control fails to influence significantly these parameters (41) in patients with diabetic nephropathy.

Recently Viberti et al. (45) have reported that patients with longstanding IDDM and urinary albumin excretion rates > 30 μg/min (or 42 mg/24 h) have a higher risk of progressive renal dysfunction 14 yr later than do IDDM patients with lower urinary albumin levels. The current studies cannot be directly compared since they represent cross-sectional analyses and since the measures of urinary albumin were less sensitive. Yet urinary albumin excretion rates > 42 mg/24 h but <400 mg/24 h were not necessarily associated with more advanced glomerular changes (Fig. 11). Conversely, some patients with glomerulopathy of a severity bordering that regularly associated with clinical nephropathy had normal UAEs. Although the present studies did not uncover any functional parameters that would predict the severity of the underlying diabetic nephropathology in patients without overt diabetic nephropathy, longitudinal investigations of structural-functional relationships in such patients will probably provide valuable information.

The mesangium and the inversely correlated peripheral capillary filtration surface area predicted hypertension with remarkable accuracy. Severe ablation of filtration surface in rats is regularly accompanied by the development of hypertension (46). Three patients with hypertension, proteinuria, and marked mesangial expansion had normal GFRs. Although preliminary, this suggests that reduced GFR is not a prerequisite to hypertension in diabetic nephropathy. It is possible that diminished filtration surface with normal or near-normal numbers of functioning nephrons can trigger adaptive mechanisms that subsume hypertension in diabetic nephropathy.

Increased cortical interstitial fibrous tissue is correlated with increased mesangial enlargement. Since mesangial expansion was related to albuminuria, GFR, and hypertension, it follows that the index of interstitial fibrosis predicted these clinical manifestations of diabetic nephropathy. The changes in the cortical interstitium, except as related to hyalinized glomeruli, were subtle even in patients with marked mesangial abnormalities. It is thus doubtful that interstitial changes could account for all of the functional disturbances that represent clinically manifest diabetic nephropathy. Nonetheless, one cannot dismiss the interstitial alterations as being unimportant (11), and more detailed studies of individual patients are required. Furthermore, the close correlation between the mesangium and the interstitium in diabetic nephropathy suggests that studies of renal interstitial cells in diabetes could prove useful.

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