Mechanisms of Progressive Glomerular Injury in Membranous Nephropathy

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Abstract. Glomerular function and structure were serially evaluated in 15 patients with membranous nephropathy who exhibited relapsing nephrosis and chronic depression of GFR. GFR declined from 56 ± 8 (mean ± SEM) at onset to 31 ± 4 ml/min per 1.73 m² after a 2- to 5-yr period of observation (P < 0.05). An analysis of filtration dynamics suggested persistent elevation of net ultrafiltration pressure. To examine a possible role for declining intrinsic glomerular filtration capacity as the basis for the observed hypofiltration, glomeruli in the baseline and a repeat biopsy (performed after a median of 28 mo) were subjected to morphometric analysis and mathematical modeling. Analysis of the baseline biopsy revealed a reduction in filtration slit frequency and thickening of the glomerular basement membrane, lowering computed hydraulic permeability by 66% compared with normal kidney donors. In contrast, filtration surface area was increased by 37% as a result of glomerular hypertrophy. The repeat biopsy revealed persistent depression of hydraulic permeability, primarily owing to foot process broadening. An additional finding was a decrease in filtration surface area from baseline in patent glomeruli, possibly due to encroachment on the capillary lumen of an increasingly widened basement membrane. Also, a striking increase in the prevalence of global glomerulosclerosis from 7 ± 2% to 23 ± 4% was found between the two biopsies, suggesting a significant loss of functioning nephrons. It is concluded that hypofiltration in membranous nephropathy is the consequence of a biphasic loss of glomerular ultrafiltration capacity, initially owing to impaired hydraulic permeability that is later exacerbated by a superimposed loss of functioning glomeruli and of filtration surface area.

A substantial minority of patients with membranous nephropathy (MN) are at risk of developing end-stage renal failure. The reported prevalence of this severe form of MN has varied widely among reporting centers and geographic regions, ranging from 20 to 40% (1–8). However, virtually all longitudinal studies of MN have identified a subset of patients who manifest azotemia at, or soon after, the outset of the glomerular injury (1–8).

The precise mechanism by which MN leads to chronic depression of GFR has not been clearly established. Serial studies of glomerular pathology in subjects with progressive azotemia have pointed to increasing curtailment of filtration surface area. One mechanism by which filtration surface area can be lowered is sclerosis of entire glomerular tufts (9–12). The degree of global sclerosis of glomeruli in azotemic subjects with MN, however, has not always been sufficient to explain the profound depression noted in GFR. This disparity suggests that the filtration capacity of patent glomeruli may also be impaired. A common finding in serial pathologic evaluations of remnant glomeruli in MN has been progressive thickening of the glomerular basement membrane (GBM), an alteration that has been posited to encroach upon the capillary lumen and thus limit the surface area available for filtration (9). We have shown earlier that progressive azotemia can also occur in the absence of a reduction in filtration surface area (13). In such cases, persistent broadening and effacement of epithelial foot processes were the most striking abnormalities. The declining rate of filtrate formation under these circumstances was attributed to impairment of the intrinsic hydraulic permeability of the glomerular capillary wall primarily due to a decrease in filtration slit frequency (13).

During the past 5 yr, we have had the opportunity to study serial renal biopsies of 15 individuals in whom MN was associated with chronic depression of GFR. To further elucidate the mechanism(s) of persistent hypofiltration, we combined a morphometric analysis of glomerular structure with mathematical modeling and a longitudinal evaluation of glomerular function. Our findings form the basis of this report.

Materials and Methods

Patients

The subjects of the present report are 15 patients with biopsy-proven MN who presented with the nephrotic syndrome and underwent a repeat renal biopsy because of persistent GFR depression.
during a 2- to 5-yr window of observation. They included 10 men and five women, and their ages varied between 21 and 62 yr. Judged by the onset of edema or first detection of proteinuria, the MN had a median duration of 8 mo (range, 1 to 13) at the time of initial evaluation. The median interval between the diagnostic biopsy and initial evaluation of renal function was 2 mo (range, 1 to 11). MN was associated with positive serology for systemic lupus erythematosus in three female members of the group, and was idiopathic in the remaining 12 subjects. Thirteen subjects received initial treatment with prednisone, which in four instances was combined with cytotoxic agents (cytoxan or imuran). The two subjects not initially treated with prednisone received monotherapy with cyclosporin A (CsA) from the outset. Prednisone was prescribed in a high dosage (approximately 1.0 mg/kg per 24 h) for 3 mo and gradually tapered thereafter. The median duration of such therapy was 5 mo (range, 2 to 12). In all cases, prednisone/cytotoxic therapy failed to induce a long-lasting remission of the nephrotic-range proteinuria. All but two patients who initially received prednisone or cytotoxic agents were given second line therapy with CsA (14). CsA was administered in an average dose of 4 mg/kg per 24 h in two divided doses, so as to maintain trough levels in serum between 50 and 150 ng/ml. The CsA was administered in 3- to 6-mo courses, each of which was followed by a 1-mo washout (14). Without exception, treatment with CsA lowered the level of proteinuria to subnephrotic levels. However, withdrawal of CsA was invariably followed by a relapse of proteinuria to levels comparable to those that preceded therapy, necessitating the reintroduction of CsA. The mean duration of CsA therapy was 14 mo (range, 3 to 33).

A follow-up biopsy was performed between 11 and 84 mo (median, 28) after the initial, diagnostic biopsy because of one of two clinical indications. One indication was to exclude transformation to a proliferative form of lupus nephritis in the three patients whose MN was associated with positive lupus serology. Each had exhibited worsening nephrosis and a steep decline in GFR despite immunosuppressive treatment. The second indication was to determine whether CsA therapy had led to a chronic renal injury, thereby precluding a further course of treatment with this agent. The repeat biopsies confirmed the persistence of MN in the three subjects with systemic lupus erythematosus, and the absence of CsA-induced chronic nephropathy in all but one of the 13 subjects who received this agent.

Two groups of healthy individuals were studied to provide control values for the glomerular functional and structural parameters. Control group 1 was composed of 104 healthy volunteers. Their ages varied between 18 and 69 yr and 66 were men. They underwent renal clearance studies identical to those performed in the patient population. Control group 2 was composed of 19 living kidney transplant donors. Their ages varied between 30 and 47 yr and seven were women. Each underwent a renal biopsy at the time of transplantation. All denied a history of renal disease, hypertension, or diabetes mellitus. At the time of evaluation, each was found to be normotensive and normoglycemic, to have a normal serum creatinine level, and to have a urinary protein excretion rate in the normal range.

**Physiologic Evaluation**

Clearance studies were performed during water diuresis between 8 a.m. and noon in our Clinical Research Center. They were undertaken at the time of presentation (baseline) and at 6- to 12-mo intervals thereafter over a 2- to 5-yr period of follow-up. During the initial clearance, no patient was receiving CsA. Follow-up clearances were performed >48 h after discontinuation of CsA therapy in those patients still receiving this agent (n = 6). Other patients had discontinued CsA 6 to 12 mo earlier or never received CsA. The 48-h interval was chosen because CsA has been shown to have a transient effect to decrease GFR and renal blood flow (maximal effect by 4 to 6 h after peak blood level). This effect disappears by 12 h after a dose (15).

In the clearance study, a priming dose of inulin (50 mg/kg) and para-aminomuographic acid (PAH; 12 mg/kg) was administered. Thereafter, inulin and PAH were given by continuous infusion to maintain constant plasma levels at 20 and 1.5 mg/dl, respectively. Sixty minutes after the priming infusion, arterial BP was determined, and blood was sampled for measurement of plasma oncotic pressure (πs) and plasma albumin concentration. Four spontaneously voided, timed urine samples were then carefully collected; each was bracketed by a blood sample drawn from a peripheral vein. GFR was expressed as the average value for the four timed inulin clearances. The rate of renal plasma flow (RPF) in the subjects with MN was determined by dividing the corresponding clearance of PAH by an estimated renal arteriovenous extraction ratio for PAH of 0.7, a value that we have found previously in nephrotic patients with depression of GFR (13,16). The PAH extraction ratio assigned to the healthy volunteers of control group 1 was 0.9 (16). The rate of albumin excretion was determined from the first of the timed urine collections. The fractional clearance of albumin was then calculated by dividing its clearance by the corresponding clearance of inulin. The concentrations of inulin and PAH were determined with an automated assay (13,16). Concentrations of albumin in serum and urine were determined by immunochemical methods and plasma oncotic pressure by membrane osmometry, as described previously (17).

**Morphometric Evaluation**

**Light Microscopy.** All glomeruli in a single, 1-μm-thick section stained with periodic acid-Schiff reagent were analyzed at the light microscopic level. On average, 14 (range, 4 to 49) glomeruli were examined in the initial biopsy and 15 (range, 5 to 30) in the repeat biopsy in the patients with MN. The average number of glomeruli among the 19 control biopsies was 19 (range, 5 to 58). A dedicated computer system (Southern Micro Instruments, Atlanta, GA), consisting of a video camera and monitor, microscope and digitizing tablet, was used to perform the measurements. The outline of each glomerular tuft in the section was traced onto the digitizing tablet at ×900 magnification, and the mean tuft cross-sectional area (Aᵣ) was determined using computerized planimetry. The measured tuft area included any parts with segmental sclerosis. We next counted the numbers of patent (Nᵣ) and globally sclerotic (Nₛ) glomeruli in a single section of cortical tissue. Serial sections were examined to verify the assignment of Nᵣ in the single section. The percentage of globally sclerotic glomeruli (Gₛ) was calculated by:

\[ Gₛ = \frac{Nₛ}{Nᵣ + Nₛ(Dᵣ/Dₛ)} \times 100, \]  

where Dᵣ and Dₛ are the mean diameters of globally sclerotic and patent glomeruli, respectively, derived from the tuft cross-sectional areas (13). The ratio Dᵣ/Dₛ accounts for the difference in the probability of encountering a glomerulus of either type in a random cross section due to their different sizes. The percentage of segmentally sclerotic glomeruli (Gₛ) was determined separately by analysis of serial cross sections of all glomerular tufts in the biopsy at 10- to 20-μm intervals. Glomeruli exhibiting sclerosis or hyalinosis in any of the cross sections were regarded as segmentally sclerotic (18,19).
Glomerular volume \( V_G \) was calculated from measured \( A_G \) as follows:

\[
V_G = \frac{\beta}{d(A_G)^{2/3}}(f_c)^{-3},
\]

(2)

where \( \beta \) is a dimensionless "shape coefficient" \((\beta = 1.38 \text{ for spheres})\), \( d \) is a "size distribution coefficient" that is used to adjust for variations in glomerular size \((20)\), and \( f_c \) is a correction factor for the tissue shrinkage associated with paraffin embedding \((21)\). We used \( d = 1.1 \) as in previous studies \((16,18)\), which corresponds to a distribution of glomerular sizes with an SD of approximately 25% of the mean size* \((20)\). The value we determined previously for the shrinkage factor \( f_c \) in our embedding procedure is 0.86. The fractional interstitial area was examined at \( \times600 \) magnification. A 10-\( \times \)-10-point grid was superimposed over each field in the entire cross section, and the fraction of total area occupied by interstitium was determined by point counting. Interstitial area was defined as that outside tubular and vascular structures, other than peritubular capillaries.

**Electron Microscopy.** For transmission electron microscopy, tissue was fixed in 2.0% paraformaldehyde in 0.1 M cacodylate buffer and embedded in epon. Toluidine blue-stained sections were then surveyed to locate blocks with patent glomeruli present entirely within the block. Except for two cases in which only one glomerulus was available, ultrastructural analysis was performed on two glomerular profiles in each patient. The glomeruli analyzed could contain segmental lesions. Ultrathin sections \((60 \text{ to } 70 \text{ nm})\) of the selected glomeruli were stained with lead citrate and uranyl acetate. A complete montage of each glomerulus at \( \times2820 \) was prepared, and line-intercept counting was used to calculate the fractional surface density at the subendothelial aspect of the peripheral capillary wall \((S_e)\) by standard stereologic methods \((20)\). Capillary luminal cross-sectional area was estimated as \( V/2L_e\). A 30-mm square test grid (equal to 10.6 \( \mu \text{m} \) on a side) was used with the prints. Six to eight images of peripheral capillary loops chosen at roughly uniform intervals around each of the glomerular profiles were then photographed at \( \times11,280 \) to evaluate the frequency of epithelial filtration slits, the thickness of the peripheral glomerular basement membrane, and the stage of injury according to the classification of Ehrenreich and Churg \((9)\). Filtration slit frequency \((\text{FSF})\) was determined by counting the total number of epithelial filtration slits and dividing it by the total length of the peripheral capillary wall at the epithelial interface \((22,23)\). The harmonic-mean basement membrane thickness \((\delta_{bm})\) was calculated for each individual, using the method of orthogonal intercepts \((24)\):

\[
\delta_{bm} = \frac{8}{3\pi} \times \delta'^{bm},
\]

(3)

where \( \delta'^{bm} \) is the apparent harmonic mean basement membrane thickness. Measured thickness included both normal GBM material and intramembranous deposits. The number of intercepts per individual was between 142 and 192 on average.

*Because the number of tuft profiles used to calculate the mean tuft cross-sectional area influences the precision of the estimate of \( V_G \) derived from the Weibel–Gomez formula \((\text{equation } 2)\), we also calculated a weighted mean \( V_G \) for each group. The weighting factors were derived from a computer analysis of the Weibel–Gomez method \((J \text{ Am Soc Nephrol } 8: \text{621A, 1997})\). The weighted group mean \( V_G \) did not differ \((<3.8\%)\) from the values shown in Figure 3.

**Calculations**

The total filtration surface area \( S \) was calculated from:

\[
S = S_v \times V_G,
\]

(4)

where \( S_v \) and \( V_G \) are the fractional surface density and glomerular tuft volume, respectively.

The intrinsic hydraulic permeability of the glomerular capillary wall \((k)\) was estimated from the FSF and basement membrane thickness \((\delta_{bm})\) by using a hydrodynamic model of viscous flow that has been described in detail elsewhere \((25)\). In this model, the capillary wall consists of a large number of repeating structural units, each of which is based on a single filtration slit. The width of such a structural unit \((W)\) is calculated from FSF by:

\[
W = \frac{2}{\pi} \times \frac{1}{\text{FSF}},
\]

(5)

where \(2/\pi\) is a stereologic factor that accounts for the random angle of sectioning.

Considering the capillary wall as a system of resistances in series, the overall hydraulic permeability is calculated from the permeabilities of each component layer by:

\[
k = \left( \frac{1}{k_{en}} + \frac{1}{k_{bm}} + \frac{1}{k_{ep}} \right)^{-1},
\]

(6)

where \(k_{en}, k_{bm}, \text{ and } k_{ep}\) are the hydraulic permeabilities of the endothelium, basement membrane, and epithelium, respectively. Many of the needed structural parameters have not been measured for the human glomerular capillary wall, requiring substitution of corresponding values derived for rats, as described in detail by us previously \((22,25)\). The values derived from previous studies in the rat and used in the model calculation include the permeabilities of the endothelium \((k_{en} = 2.0 \times 10^{-7} \text{ m/s per Pa})\) and of the slit diaphragm \((k_s, 7.9 \times 10^{-8} \text{ m/s per Pa})\), the filtration slit diaphragm width \((W_s, 41 \text{ nm})\), and the Darcy permeability of the GBM \((K_G, 2.7 \text{ mm}^2)\) \((25)\). A preliminary study in our laboratory has shown that the value for \(W_s\) in humans is probably quite similar \((36 \pm 4 \text{ nm}, n = 4)\) and does not appear to differ between patients and healthy kidney donors. As mentioned above, the values for FSF and \(\delta_{bm}\) needed for the model were derived from individual MN patients and normal donors.

The permeability of the epithelial layer was calculated using:

\[
k_{ep} = \frac{\varepsilon}\varepsilon W_s k_s W_s k_s,
\]

(7)

where \(\varepsilon\) is the fraction of the basement membrane area occupied by filtration slits, and \(W_s\) is the slit width \((\varepsilon = W_s/W)\). The permeability of the basement membrane \((k_{bm})\) was calculated using equation 21 of Drumond and Deen \((25)\).

The single nephron ultrafiltration coefficient \((\text{SNK})\) was calculated from the product of filtration surface area \((S)\) and the hydraulic permeability of the walls of patent glomerular capillaries \((k)\) in the glomeruli, which were examined ultrastructurally. In making this calculation, we corrected for the effect of immersion fixation to decrease glomerular dimensions relative to the situation in situ \((21)\).

**Statistical Analyses**

Results are expressed as means ± SEM except for those related to urinary protein excretion and albumin clearance which, because of their skewed distributions, are expressed as medians (and range). The Behrens–Fisher \(t\) test and a Bonferroni correction were used to test the significance of differences between the early and late findings in the
patients with MN and corresponding values in healthy control subjects. Depending on whether their distribution was Gaussian or non-Gaussian, a paired t test or Wilcoxon test was used to test the significance of changes in glomerular function or structure in the MN group over time. Linear regression was assessed by the method of least squares.

Results

Glomerular Function

Glomerular function at the initial and final evaluation over the 2- to 5-yr window of observation in the patients with MN is summarized in Table 1 and Figure 1. Proteinuria remained in the nephrotic range at the end of the observation period in 13 of the 15 subjects with MN, with the result that neither the level of proteinuria nor the fractional albumin clearance was significantly different from the corresponding baseline values (Table 1). GFR at the time of presentation varied widely, ranging from values that were markedly depressed to values within the normal range (Figure 1). On average, the baseline GFR was 56 ± 8, significantly less than the normal control value of 102 ± 2 ml/min per 1.73 m² (P < 0.001). The baseline RPF averaged 585 ± 72 ml/min per 1.73 m², a value not significantly different from normal. However, there was also marked variability in RPF such that observed values ranged from depressed to supernormal (153 to 925). Selective elevation of RPF or a proportionately smaller decline in RPF than corresponding GFR resulted in marked depression of the baseline filtration fraction (9 ± 1 versus 19 ± 1% in control subjects). Reflecting the massive urinary protein losses (Table 1), the oncotic pressure of plasma entering the glomerular tufts (πA) was markedly depressed: 11 mmHg below the control value at the onset of the MN. In contrast, mean arterial pressure (MAP) was elevated, exceeding the control value by 18 mmHg (Table 1).

GFR at final examination was depressed below normal in each subject with MN (Figure 1). On average, it declined from the initial value of 56 ± 8 to 32 ± 4 ml/min per 1.73 m² (P < 0.05). Note that whereas GFR declined in 11 individuals, it was constant or increased slightly in the remaining four individuals. However, each of the latter exhibited marked hypofiltration at initial examination, and the GFR in each instance failed to return to the normal range during the period of observation. Although RPF also tended to decline between the first and last examination, there was a parallel decline in GFR, with the result that the filtration fraction remained depressed. The πA was also persistently depressed at the final examination, whereas arterial pressure remained elevated (Table 1). As a result, the net pressure for ultrafiltration is likely to have been elevated at both examinations.

Glomerular Morphometry

Findings at the light microscopic level are illustrated in Figure 2. Segmental glomerulosclerosis was prominent at both

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Table 1. Glomerular function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Baseline</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>102 ± 2</td>
<td>56 ± 8b</td>
<td>32 ± 4b,c</td>
</tr>
<tr>
<td>RPF (ml/min per 1.73 m²)</td>
<td>561 ± 13</td>
<td>585 ± 72</td>
<td>392 ± 59d,e</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>19 ± 1</td>
<td>9 ± 1b</td>
<td>10 ± 0.9b</td>
</tr>
<tr>
<td>Oncotic pressure (mmHg)</td>
<td>24 ± 0.3</td>
<td>12.7 ± 1b</td>
<td>16 ± 1.4c,f</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>90 ± 1</td>
<td>108 ± 2.8b</td>
<td>112 ± 3.5b</td>
</tr>
<tr>
<td>FC albumin (10⁻⁵)</td>
<td>0.25</td>
<td>1318b</td>
<td>604b</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>ND</td>
<td>(0.66 to 1.75)</td>
<td>(25 to 5149)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25 to 5149)</td>
<td>(43 to 7328)</td>
</tr>
</tbody>
</table>

* RPF, renal plasma flow; FC albumin, fractional clearance of albumin; ND, not detectable by dipstick.

b P < 0.001, membranous nephropathy versus controls.

c P < 0.01, final versus baseline.

d P < 0.05, membranous nephropathy versus controls.

e P < 0.05, final versus baseline.

f P < 0.01, membranous nephropathy versus controls.
examinations, involving 48 ± 8 and 44 ± 7% of glomeruli in the baseline and repeat biopsies, respectively (left panel). In contrast, the prevalence of global glomerulosclerosis was low at baseline, affecting only 7 ± 2% of glomeruli. With the passage of time, the percentage of glomeruli exhibiting global sclerosis increased in most patients, to 23 ± 4% (P < 0.01, middle panel). Increasing global sclerosis was accompanied by marked expansion of the interstitial compartment. The fractional interstitial area increased significantly from a modestly elevated value in the baseline biopsy to one that was strikingly elevated at the time of repeat biopsy, 23 ± 3 versus 38 ± 3%, respectively (P < 0.001, right panel). Of note, the interstitial expansion was generalized and not of the focal or "striped" pattern observed with CsA-induced chronic renal injury (26).

Only a single instance of medial necrosis and hyalinosis of an afferent arteriole was observed among the 15 repeat biopsies. The absence of striped interstitial fibrosis and paucity of afferent arteriopathy suggest that CsA therapy per se did not make an important contribution to the observed hypofiltration (26).

The morphometric analysis of filtration surface area and its determinants in patent, remnant glomeruli is illustrated in Figure 3. The remnant glomerular volume at baseline in MN was twofold larger than the corresponding control value, 3.6 ± 0.4 versus 1.8 ± 0.1 μm³ x 10⁶, respectively (P < 0.001). There was a small, statistically insignificant decrease in the volume of remnant glomeruli in the repeat biopsy (3.1 ± 0.4 μm³ x 10⁶, left panel). Filtration surface density was only modestly depressed at baseline, but was shown to have declined sharply at the time of repeat biopsy, 0.10 ± 0.004 versus 0.06 ± 0.005 μm²/μm³, respectively (P < 0.001, middle panel). From the product of glomerular volume and filtration surface density, we conclude that filtration surface area was numerically larger than the control value at baseline, but decreased by half in the repeat biopsy, 3.7 ± 0.4 versus 1.8 ± 0.3 μm² x 10⁶, respectively (P < 0.01, right panel). Especially noteworthy is the consistent decrease in filtration surface density.

The morphometric analysis of the determinants of the intrinsic hydraulic permeability is illustrated in Figure 4. The GBM in remnant glomeruli at baseline was thickened in 12 of 15 MN subjects; mean GBM thickness exceeded the control value almost twofold, 741 ± 66 versus 403 ± 15 nm, respectively (P < 0.001). Each subject exhibited a further increase in GBM thickness with the passage of time. As a result, GBM thickness in the repeat biopsy (1592 ± 125 nm) was twice the baseline value (P < 0.001, left panel). This was partly the result of an increase in extracellular matrix. Particularly striking, however, was a dramatic increase in the number and size of subepithelial

![Figure 2](image1.png)  
*Figure 2.* Morphometric findings in baseline and repeat biopsies in 15 subjects with MN. The percentage of glomeruli exhibiting segmental sclerosis (SS) or global sclerosis (GS) are in the left and middle panels, respectively. Fractional interstitial area (FIA) is in the right panel. The remaining conventions are as in Figure 1. *P < 0.01; **P < 0.001.

![Figure 3](image2.png)  
*Figure 3.* Morphometric determinants of filtration surface area in remnant glomeruli. Glomerular volume (Vg) and filtration surface density (Sv) are in the left and middle panels, respectively. Computed filtration surface area (S) is in the right panel. The remaining conventions are as in Figure 2.
and intramembranous electron-dense deposits (Figure 5). According to the Churg classification, the baseline biopsy was stage I in two patients, stage II in eight patients, and stage III in five patients. Corresponding findings in the repeat biopsy were stages II, III, and IV in two, 11, and two patients, respectively. Thus, during the observation period, disease severity advanced in a majority of patients.

As can be seen in Figure 5, the increasingly thickened capillary wall appears to encroach on the lumen in the repeat biopsy, and provides a likely basis for the observed reduction in filtration surface density. In fact, average luminal cross-sectional area declined by 15%, from 62.3 ± 6.2 to 46.8 ± 3.6 \( \mu m^2 \) \((P \approx 0.02)\) between the initial and follow-up biopsies. Interestingly, as noted qualitatively by Ehrenreich and Churg almost 30 yr ago (9), there was a tendency for the capillary lumina in MN in the initial biopsy to be larger than in control subjects (53.4 ± 3.4 \( \mu m^2 \)). Foot process broadening was also a uniform finding in the baseline biopsy, and resulted in a substantial decrease in the filtration slit frequency compared with control, 377 ± 45 \( \text{versus} \) 1324 ± 42 slits/mm, respectively \((P < 0.001, \text{middle panel, Figure 4})\). In contrast to GBM thickness, however, filtration slit frequency did not change between the two biopsies, averaging 489 ± 41 slits/mm in the repeat biopsy. Reflecting the low but constant value for filtration slit frequency in the two biopsies, computed hydraulic permeability remained low, increasing only slightly from its initial value of 0.79 ± 0.09 to 0.85 ± 0.08 m/s per Pa \( \times 10^{-9} \). Both values are significantly lower than the corresponding control value of 2.8 ± 0.09 m/s per Pa \( \times 10^{-9} \) (right panel, Figure 4).

Figure 5. Comparison of changes in glomerular capillary wall morphology between baseline (A) and repeat biopsies (B) in a representative subject with MN. Photographs at each time are at the same magnification (\( \times 11,400 \)). Bar, 3 \( \mu m \) (shown in A).
Computed Single Nephron $K_f$

Remnant single nephron $K_f$, the product of filtration surface area and hydraulic permeability, is illustrated in Figure 6. As a result of the disparate trends for filtration surface area and hydraulic permeability in the baseline biopsy, initial single nephron $K_f$ in MN varied widely. On average, it was depressed below the corresponding control value, however, 3.4 ± 0.7 versus 7.1 ± 0.6 nl/(min · mmHg), respectively ($P < 0.001$).

Although filtration surface area declined between the baseline and repeat biopsies, an offsetting increase in hydraulic permeability at the time of repeat biopsy resulted in a modest increase in single nephron $K_f$ in four individuals. In the remaining 11 subjects, however, declining surface area and a relatively constant hydraulic permeability resulted in a measurable decline in single nephron $K_f$. On average, the computed value at the time of repeat biopsy was 1.8 ± 0.4 nl/(min · mmHg), only 25% of the control value (Figure 6). In fact, as seen in Figure 7, there was a significant linear relationship ($P = 0.005$) between the change in insulin clearance and the change in calculated $SNK_f$ in the MN patients over the observation period. Regression analysis suggests that at least 50% of the variance in GFR could be explained by the change in $SNK_f$ (approximately 80% if the single apparent outlier is excluded). The regression analysis did not include one of the 15 MN patients in whom GFR was zero at follow-up (end-stage renal disease).

Discussion

In the group of patients with severe MN and relapsing nephrotic syndrome that we studied, there was a sustained or progressive depression of GFR over the course of the observation period. To understand this hypofiltration, we should consider separately the determinants of glomerular filtration, which may be broadly divided into the net transcapillary pressure favoring ultrafiltration and the total capacity of the glomerulus to form filtrate (as reflected in the ultrafiltration coefficient $K_f$).

Our physiologic assessment suggests that the observed depression of GFR in the 15 subjects of the present study is unlikely to have been due to a reduction in net ultrafiltration pressure. From the measured values for $\pi_A$ and filtration fraction (Table 1), we estimate that the glomerular capillary oncotic pressure was decreased both at baseline and at the final examination (17), an alteration that, by itself, should have led to an enhanced rather than a diminished ultrafiltration pressure (27). It is, of course, theoretically possible that the net pressure for ultrafiltration was decreased by a fall in the transcapillary hydraulic pressure gradient ($\Delta P$), a parameter that cannot be measured in humans. Such a depression of $\Delta P$ would have to be substantial, however, to offset the decline in glomerular capillary oncotic pressure (28). There are two reasons that lead us to believe that a decline in $\Delta P$ of this magnitude in the subjects of the present study is highly improbable. One is that micropuncture studies of rat analogues of MN have invariably revealed an increased $\Delta P$ compared with control subjects (28–30). The other reason is that even if afferent vascular resistance were increased in MN, it is hard to conceive how $\Delta P$ could have been depressed given the quite significant elevations of mean arterial pressure that were observed at baseline and follow-up in the subjects of our study (Table 1). Transmission of even a minor fraction of this pressure elevation to the glomerular capillaries should have increased and not reduced $\Delta P$, thereby enhancing the net pressure for ultrafiltration (27).

The foregoing observations lead us to infer that a decline in net ultrafiltration pressure is unlikely to have contributed significantly to the chronic hypofiltration in our patients, and that a loss of ultrafiltration capacity of the glomerular capillary walls is the principal cause of this phenomenon. Analysis of the baseline biopsies points to severe impairment of hydraulic permeability as the predominant factor early on, i.e., during the months that followed the onset of the glomerular injury
This reflects mostly a striking broadening of epithelial foot processes with an ensuing reduction in the frequency of epithelial filtration slits (22). Impaired intrinsic hydraulic permeability is offset to some extent by glomerular hypertrophy, which serves to enhance filtration surface area in the large majority of glomeruli that remain patent early in MN (Figure 2). Analysis of repeat biopsies performed a median of 28 mo after the baseline biopsy reveals a second phase, one in which a progressive loss of filtration surface area is superimposed on persistently impaired hydraulic permeability in the patent glomeruli (Figures 3 and 4). Also contributing to the depression of GFR in this phase is a dramatic increase in the number of globally sclerotic glomeruli (Figure 2).

In a landmark study of serial glomerular histopathology almost 30 yr ago, Ehrenreich and Churg first pointed out that progressive azotemia in MN was associated with marked thickening of the GBM (9). This finding has since been confirmed by others (10–12) and was invoked by Ehrenreich and Churg to explain progressive hypofiltration on the basis of encroachment by the GBM on the capillary lumen with an ensuing curtailment of the peripheral capillary wall area available for filtration. Our analysis of repeat biopsies confirms that filtration surface density (S,) is indeed lowered and that progressive encroachment on the capillary lumen by the increasingly thickened capillary wall does occur.

CsA is known to decrease GFR, and 13 of our patients received CsA at some time during this study, raising the possibility of a role for CsA therapy in the persistent hypofiltration seen in our patients. In the repeat biopsy, however, neither subintimal hyalinosis nor medial necrosis was observed infferent arteriolar walls of 12 of the 13 subjects treated with CsA (26,31). Thus, pathologic evidence of CsA nephrotoxicity at the time of the repeat biopsy is lacking. In addition, only six patients were taking CsA at the time of the follow-up evaluation of renal function; in these, CsA was withheld long enough to avoid its known transient effects on GFR (15).

We wish to emphasize that the precision of our computed values for the intrinsic hydraulic permeability of the glomerular capillary is limited by the need to rely on several assumptions. For example, our ultrastructural model requires extrapolation from the rat of several dimensional parameters of the glomerular capillary wall (25). These include the dimensions of endothelial fenestrae and the width of the filtration slit diaphragms. Preliminary data from our laboratory suggest that filtration slit diaphragm widths in our patients did not differ significantly from the values determined in rats. Another limitation of the model is the need to assume that the Darcy permeability of the human GBM is the same as that which has been measured in the rat in vitro (32). We performed sensitivity analyses to set limits on the potential errors in our computation of hydraulic permeability. These analyses suggest that despite the uncertainties introduced by the aforementioned assumptions, such errors are unlikely to be large (22). Furthermore, the accuracy of determinations of hydraulic permeability from our model has been verified in the rat by the demonstration of their agreement with values for k determined from single-nephron K, measured by micropuncture and morphometrically measured values for S (25). Such studies in the healthy rat indicate that the GBM and filtration slit each account for about half of the resistance to water flow. Moreover, although thickening of the GBM and reduction of filtration slit frequency are each predicted to lead to a lowered k (25), in the context of a low filtration slit frequency the model predicts a decreased influence of k, on total hydraulic permeability. This is because with fewer filtration slits most of the water flow within the GBM is parallel to the membrane, and the effect of the additional path length across a thickened GBM becomes trivial (22). This explains our finding that despite a doubling of GBM thickness, there was a negligible effect on the computed value for k at the follow-up biopsy (Figure 4). The effect of deposits in the GBM on k, is quite difficult to predict, but may similarly be of lesser importance in the context of a decreased filtration slit frequency.

Our computation of effective filtration surface area is also subject to error. This determinant of SNK, requires knowledge of the glomerular volume, the determination of which is subject to artefacts associated with both immersion fixation and paraffin embedding (21). Although we introduced correction factors for these artefacts, the ensuing glomerular shrinkage is likely to vary from biopsy to biopsy, and the use of a single correction factor could limit the accuracy with which glomerular volume has been determined in each individual. Yet another confounding factor is the small number of glomeruli that we were able to examine in some of these clinical biopsy specimens. The effect of the latter on the estimation of VG has been shown by a computer analysis of the Weibel–Gomez method to be trivial (≤3.8%) when compared with the magnitude of the difference in VG between MN and control groups. In fact, the major factor accounting for the steep decline in SNK, found over the observation period was the decrease in filtration surface density, a parameter unaffected by the above-mentioned considerations.

Despite these potential limitations, we found a highly significant correlation between the decline in GFR (a completely functional parameter) and the decline in calculated single nephron K, (a morphometric and model-based parameter) in our patients with MN. In our view, this is convincing evidence of the validity of a morphometric approach to the assessment of K, in humans and corroborates the close agreement we found earlier between morphometrically determined and micropuncture-derived values for K, in the rat (25).

The present study was observational in nature. It was made possible by the availability of a repeat renal biopsy that was performed for clinical indications. Inasmuch as we have not studied glomerular morphology in MN patients who exhibited remission of nephrosis and a normal GFR, our observations are uncontrolled. We cannot exclude the development of similar structural changes in such patients, despite their favorable outcome. The findings in a number of earlier reports of serial biopsies in MN make this unlikely, however. For example, in serial histopathologic studies of 44 patients who were biopsied after their proteinuria had resolved completely (10–12,33–36), the glomeruli in many such cases were shown to have reverted to an appearance that was entirely normal (11). Glo-
meruli of most remaining subjects exhibited mild persistent abnormalities that included only focal GBM thickening and areas of electron lucency, inferred to represent reabsorbed immune deposits (10–12,33–36). Foot processes were observed to be discrete for the most part, or broadened only in a patchy manner. These observations suggest that hydraulic permeability is unlikely to have been substantially impaired (22,25).

We conclude that sustained or progressive hypofiltration in MN after 2 to 5 yr of follow-up is attributable to a striking biphasic loss of ultrafiltration capacity. Our conclusion is based on the fact that the decrement in GFR in these patients is quantitatively accounted for by the decrease in single-nephron \( K_f \) (determined morphometrically) and the measured increase in global sclerosis. We submit that given the large magnitude of the underlying changes in glomerular structure over a relatively short period of follow-up, detailed morphometric analysis could provide a sensitive measure by which to judge the efficacy of future potential therapies in this disorder.

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References