Role of Abnormally High Transmural Pressure in the Permselectivity Defect of Glomerular Capillary Wall: A Study in Early Passive Heymann Nephritis

Toshimasa Yoshioka, Helmut G. Rennke, David J. Salant, William M. Deen, and Iekuni Ichikawa

To explore the mechanism of glomerular permselectivity defect in passive Heymann nephritis, an experimental model of human membranous glomerulopathy, Munich-Wistar rats were subjected to both microcuvure assessment of glomerular hemodynamics and whole kidney clearance measurements of graded size dextrans 10 days after injection of sheep anti-rat tubular antigen (anti-Fx1A). Compared with normal control rats, anti-Fx1A-treated animals were characterized by marked proteinuria (65±8 μg/min versus 6±1, p<0.001), markedly and significantly higher glomerular transcapillary hydraulic pressure difference (40±1 mm Hg versus 33±1, p<0.001), depressed ultrafiltration coefficient and impaired glomerular size-selective function as determined by fractional clearance of dextrans. Calculation of membrane parameters based on a recently defined heteroporous membrane model revealed abnormally high availability of non-size-selective, large pore pathways in the glomerular capillary wall of the rats with passive Heymann nephritis. To ascertain the role of the altered hemodynamic pattern in the observed defect in the size-selective function of the glomerular capillary wall, glomerular transcapillary hydraulic pressure difference was manipulated experimentally in these proteinuric rats by intra-aortic infusion of acetylcholine or angiotensin II. These agents respectively suppressed and augmented glomerular transcapillary hydraulic pressure difference and brought about a decline of and a further rise in fractional clearance of larger dextrans along with parallel changes in both urine protein excretion rate and availability of nonselective channels. These results indicate that the permselectivity defect in passive Heymann nephritis is attributable, at least in part, to impaired size selectivity of the glomerular capillary wall caused by a prevailing abnormally high transcapillary hydraulic pressure difference. (Circulation Research 1987;61:531–538).

Hemodynamic factors can affect the glomerular filtration of macromolecules in two ways. First, alterations in single nephron glomerular filtration rate (SNGFR) or its determinants, including glomerular plasma flow rate (Q_0) and mean glomerular transcapillary hydraulic pressure difference (∆P), may modify the convection and diffusion of solutes across the capillary wall, even if the intrinsic selectivity of the barrier to molecules of varying size and charge remains unchanged. This results from the influence of SNGFR and its determinants on solute concentration variations along glomerular capillaries as well as from changes in the bulk fluid flow of ultrafiltrate across the capillary wall. Second, hemodynamic perturbations may lead to alterations in the barrier structure itself, as reflected by changes in the apparent pore-size distribution and/or alterations in the barrier structure itself, as reflected by changes in the apparent pore-size distribution and/or concentration of fixed negative charges on the capillary wall. Ablation of ~85% of renal mass in the rat, which led to large increases in Q_0 and ∆P and a doubling of SNGFR one week following surgery, also resulted in defects in both the size- and charge-selective properties of the capillary wall. What appears to be hemodynamically mediated changes in glomerular size selectivity has also been demonstrated acutely. Volume expansion using hyperoncotic albumin in humans enhanced the fractional clearances of very large dextran in both normal and nephrotic individuals.

A very selective increase in the fractional clearances of large dextran, and a marked proteinuria, were observed during renal vein constriction in the rat, which acutely elevated ∆P. Attenuation of the high ∆P by pharmacologic blockade of endogenous angiotensin II action resulted in a diminution of urine protein excretion rate and partial improvement of glomerular size-selective function. Of interest is the fact that several experimental forms of glomerular injury, i.e., models for human protein-
Munich-Wistar rats (Groups 2, 3, and 4 defined below) by a single intravenous injection of 1.5 ml of sheep antiserum to rat proximal tubule epithelial antigen, Fx1A. Fx1A was isolated from Sprague-Dawley rat renal cortices in the manner described by Edgington et al and emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The antiserum to Fx1A was obtained from sheep given four monthly intramuscular injections of 75 to 100 mg of rat Fx1A. Details of collection and isolation of anti-Fx1A are given elsewhere. Ten days after the injection of either 0.9% NaCl (Group 1) or anti-Fx1A (Groups 2, 3, and 4), rats were subjected to study.

Experimental Protocols

All rats were anesthetized with Inactin (100 mg/kg body wt i.p.) and surgically prepared in a fashion routinely performed in our laboratory. Briefly, following tracheostomy, indwelling polyethylene catheters (PE-50, Clay Adams, Parsippany, N.J.) were placed into the left and right jugular and left femoral veins for subsequent infusion of plasma, whole blood, inulin, para-aminohippurate, and dextran solution. The left femoral artery was also catheterized for monitoring mean arterial pressure (MAP) and for periodic blood collections. MAP was measured by an electronic transducer (model p23Db, Gould Inc., Cleveland, Ohio) connected to a recorder (model 2200S, Gould Inc.). Laparotomy was then performed. The left ureter was catheterized with a polyethylene tube (PE-10) for subsequent urine collections, and the left kidney was immobilized for micropuncture studies. A 30-gauge needle was inserted into the abdominal aorta just above the origin of the left renal artery for the infusion of 0.9% NaCl solution at a rate of 1.2 ml/hr. To maintain the circulating plasma volume at normal euvolemic level during the experiment, each rat received isoncotic rat plasma in a volume of 10 ml/kg intravenously over the initial 30 minutes followed by continuous infusion at the rate of 0.6 ml/kg/hr. For the estimation of GFR, SNGFR, and whole kidney effective plasma flow rate (RPF), 9% inulin and 0.8% para-aminohippurate in 0.9% NaCl solution was infused intravenously with a priming dose of 0.4 ml, followed by constant infusion at a rate of 1.2 ml/hr. Following the above preparatory procedures, rats were subjected to micropuncture and dextran clearance studies specified below. In Groups 3 and 4, an attempt was made to manipulate the glomerular transcapillary hydraulic pressure difference experimentally. Thus, through the needle inserted into the abdominal aorta, 6 rats (Group 3) were given acetylcholine chloride (Sigma Chemical Co., St. Louis, Mo.) continuously at a rate of 0.35 mg/kg/hr, and 8 animals (Group 4) were given angiotensin II (Asn1, Ile4-Angiotensin II, Sigma) at a rate of 10 ng/kg/min. Following a 30-minute equilibration period, with MAP having reached a steady-state level, micropuncture and dextran clearance studies were performed.

Materials and Methods

Induction of Rat Model of Membranous Glomerulopathy

As a normal control group (Group 1), 7 Munich-Wistar rats received 1.5 ml of 0.9% NaCl intravenously and were studied 10 days later in a fashion identical to the other groups defined and specified below. Passive Heymann nephritis was induced in 26

Table of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>MAP</td>
<td>Mean arterial pressure, mm Hg.</td>
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<tr>
<td>P</td>
<td>Mean pressure, mm Hg.</td>
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<tr>
<td>ΔP</td>
<td>Mean transcapillary hydraulic pressure difference, mm Hg.</td>
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<tr>
<td>PHN</td>
<td>Passive Heymann nephritis.</td>
</tr>
<tr>
<td>π</td>
<td>Colloid osmotic pressure, mm Hg.</td>
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<tr>
<td>QA</td>
<td>Initial glomerular plasma flow rate, nl/min.</td>
</tr>
<tr>
<td>R</td>
<td>Resistance to blood flow, mm Hg/nl/min.</td>
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<tr>
<td>r0</td>
<td>Effective radius of small pores, Å.</td>
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<tr>
<td>RPF</td>
<td>Whole kidney effective plasma flow rate (whole kidney para-aminohippurate clearance), ml/min.</td>
</tr>
<tr>
<td>S</td>
<td>Total capillary surface area per glomerulus.</td>
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<td>SNFF</td>
<td>Single nephron filtration fraction.</td>
</tr>
<tr>
<td>SNGFR</td>
<td>Single nephron glomerular filtration rate, nl/min.</td>
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<tr>
<td>UprV</td>
<td>Whole kidney effective plasma flow rate, nl/min.</td>
</tr>
<tr>
<td>ωm</td>
<td>Membrane parameter related to the presence of large, nonselective pores.</td>
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<tr>
<td>η</td>
<td>Viscosity of glomerular filtrate.</td>
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Subscripts

- A: Femoral artery or afferent arteriole.
- E: Efferent arteriole.
- GC: Glomerular capillary.
- T: Proximal tubule.

uric condition, studied in recent years uniformly have been shown to have high ΔP values. Moreover, a recent study by Shemesh et al in human membranous glomerulopathy suggested enhanced availability of nonselective pathways across the glomerular capillary wall. The present study was undertaken to evaluate the role of hemodynamic factors in the abnormalities associated with the sieving function of glomeruli with immune injury. For this purpose, experimental protocols were designed to manipulate ΔP pharmacologically in passive Heymann nephritis (PHN).
Micropuncture Measurements

Using the method previously described in detail,10-12 micropuncture measurements and collections were made in all animals to determine SNGFR, mean glomerular capillary (Pp), proximal tubule hydraulic pressure (P), femoral arteriolar (C) and efferent arteriolar (C) plasma protein concentrations, single nephron filtration fraction (SNFF), initial capillary plasma flow rate (Qc), and ultrafiltration coefficient (Ku), as well as resistances of single afferent (Rs) and efferent (Re) arterioles. Colloid osmotic pressure (π) of plasma entering and leaving glomerular capillaries was estimated from Cc and Ce by using the equation derived by Deen et al.10

In addition to these micropuncture measurements, timed urine samples were taken at each study period for measurements of GFR, RPF, and urine protein excretion rate (UpV) expressed as micrograms of protein per minute. Also, for this purpose, the blood samples were taken from the femoral arterial catheter at the beginning and the end of each experimental period. These measurements were completed within approximately 20 minutes and were followed immediately by the dextran clearance measurement specified below.

Dextran Clearance Measurements

Fractionated dextran with average molecular weight of 10 (T-10), 40 (T-40), and 70 (T-70) K daltons were purchased from Pharmacia Fine Chemicals, Piscataway, N.J. A 20-mg/ml solution containing 50.0% T-70, 37.5% T-40, and 12.5% T-10 was oxidized with NaI, at pH 5.0 and reacted with tyramine in a borate buffer at pH 9.0. The tyraminil-dextran was separated from unbound reactants by gel filtration on a Sephadex T-70, 37.5% T-40, and 12.5% T-10 was oxidized with NaI, at pH 5.0 and reacted with tyramine in a borate buffer at pH 9.0. The tyraminil-dextran was separated from unbound reactants by gel filtration on a Sephadex G-25 column [total volume (VT) 42 ml] (Pharmacia Co., Rockford, Ill.) and from unbound reactants by gel filtration on a Sephadex G-25 column (VT) 42 ml (Pharmacia Fine Chemicals). The purified mixture was radiolabelled at pH 7.4 with IODO-BEADS (Pierce Chemical Co., Rockford, Ill.) and 121I (New England Nuclear, Boston, Mass.). Unreacted 121I was removed by a gel chromatography on a short disposable Sephadex G-25 column (VT, 10 ml). The ionic strength and pH of the solution were adjusted, and the mixture was sterilized by filtration and stored at 4° C. The iodinated dextran solutions were used within 7 days of the date of preparation.

The dextran clearance study was performed immediately following the micropuncture procedures. At the beginning of each dextran clearance measurement, the transit time was determined by intravenous injection of 0.2 ml of lissamine green solution. In each study period, 0.2 ml of the 121I dextran solution (=40,000 cpm/μl) was injected intravenously, followed by a constant infusion at the rate of 0.034 ml/min. Approximately 5 minutes after completion of the priming injection, a continuous collection of blood was started from the femoral artery for 20 minutes at a rate of 0.034 ml/min using a withdrawal pump (model 941, Harvard Apparatus Co., South Natick, Mass.). Urine was collected from the catheter inserted into left ureter for an equivalent time period, corrected for the transit time. The blood samples were centrifuged immediate-ly, and plasma and urine aliquots were separated for subsequent analysis.

The fractionation of urine and plasma samples containing dextrans of graded molecular size and the measurement of radioactivity of each size of dextran were performed as previously described.14,15 Briefly, separation of dextrans into narrow (2 Å) fractions was accomplished by a gel permeation chromatography using a column (VT, 194 ml, length 64 cm) packed with an even mixture of acrylamide-agarose, AcA 34 and 44 (LKB Instruments Inc., Paramus, N.J.). Radioactivity of each fraction of dextran (=1.6 ml) was then measured in a gamma counter (Biorad gamma II, Beckman Instruments, Irvine, Calif.).

Analytical Methods

Plasma and urine concentrations of inulin were determined by the macroanthrone method16 and those of PAH by the method of Bratton and Marshall modified by Smith et al.17 Details of the analytical procedures for inulin determination in nanoliter samples and Cc and Ce measurements are given elsewhere.16,17 Urine protein concentration was measured by the Coomassie brilliant blue method.18

Calculations and Statistics

Based on the data obtained from micropuncture study, SNGFR, SNFF, PA, QA, Q, Re, ∆P, and K, were calculated using equations previously published.16,17 Fractional clearances of graded size dextrans (θ) were calculated using the equation:

\[ \theta = \frac{(U/P)_{dextran}}{(U/P)_{inulin}} \]

where (U/P)dextran and (U/P)inulin refer to the urine-to-plasma ratio of dextrans and inulin, respectively.

To assess the membrane permeability characteristics for macromolecules, two membrane parameters defined by a recent mathematical model were calculated.19 This model postulates two functionally distinct pathways for movement of macromolecules across the capillary wall. One of the pathways has small pores of radius r, which dominate in number and serve as the major pathway for water and small molecules, while the other pathway contains pores that are few in number but sufficiently large in radius so that they exhibit negligible selectivity even for the largest dextrans studied (molecular radius 60 Å). The contribution of the nonelective "shunt" pathway is characterized by a parameter ω, which is related to the fraction of filtrate volume passing through these large pores. Specifically, ω is the theoretical fraction of volume flow that would take place through the large pores if all pores experienced the same net driving pressure. The actual fraction of filtrate volume passing through the postulated shunt (ω) is larger than ω, and varies with position along the capillary because flow through the small pores is opposed by the colloid osmotic pressure of capillary plasma (protein reflection coefficient near unity), whereas the filtrate through the large pores is not (reflection coefficient near zero). Local colloid osmotic...
pressure, in turn, is modulated by $\Delta P$, $Q_A$, and other hemodynamic parameters. Thus, while values for $\omega$ are affected by these parameters, $\omega_0$ is independent of these and reflects solely the characteristics of capillary wall per se.$^{19}$ The computation of $r_A$ and $\omega_A$ were performed using mean values of $C_A$, $Q_A$, $\Delta P$, $K_r$, and $\theta$ of each group. The mathematical details of the model used, and the assumptions on which it is based, are presented in detail elsewhere.$^{19}$

Statistical analyses between study groups were performed initially by one-way ANOVA followed by multiple comparisons by the Bonferroni method.$^{20,21}$ Statistical significance was defined as $p<0.05$. All values are expressed as mean $\pm$ SEM.

Results

Micropuncture Studies

Average values for body weight, MAP, and several pertinent measurements of whole kidney function obtained in each study group are summarized in Table 1 and those of single nephron function in Table 2. As shown in Table 1, while Group 1 (normal controls) and Group 2 (PHN rats) had similar values in MAP, GFR, and RPF, a marked increase in the values of UprV was noted in Group 2 rats, averaging $65 \pm 8 \mu g/min$ (versus $6 \pm 1 \mu g/min$ in Group 1). In keeping with the tendencies seen at the whole kidney level, values for both SNGFR and $Q_A$, in Group 2 (Table 2) were not significantly different from those of Group 1 control rats. Marked abnormalities, however, were noted in other microcirculatory parameters of Group 2 rats. Thus, Group 2 rats had significantly elevated $P_{oc}$, averaging $55 \pm 1$ mm Hg, compared with those in Group 1 (49 $\pm$ 1 mm Hg, $p<0.01$). Likewise, values of $\Delta P$ were elevated over Group 1 control levels ($40 \pm 1$ mm Hg, $p<0.005$). In addition to these abnormal levels of pressures, Group 2 animals were characterized by having values for $K_r$ substantially depressed, averaging $2.88 \pm 0.36$ nl/(min/mm Hg), a value less than 50% of the average obtained in Group 1 control [6.30 $\pm$ 0.08 nl/(min/mm Hg), $p<0.001$]. The high $P_{oc}$ values in Group 2 resulted from a preferential elevation in $R_2$ in this group. These patterns of glomerular microcirculatory dynamics observed in Group 2 animals duplicate the results obtained by us earlier$^{23}$ and subsequently by others$^{23}$ in rats with PHN.

During administration of acetylcholine in Group 3 rats, values for total GFR and SNGFR essentially remained at the levels identical to those of Group 2 animals. In contrast, values for MAP, $P_{oc}$, and $\Delta P$ of Group 3 were shown to be substantially depressed below the levels of Group 2. Values for both RPF and $Q_A$ were higher in Group 3 than Group 2, on average by some 20% (only RPF reached statistical significance) (Tables 1 and 2). Calculation of arteriolar resistances revealed that values for both $R_A$ and $R_2$ in Group 3 were depressed below the Group 2 levels.

| Table 1. Summary of Data for Whole Body and Kidney Function in Four Experimental Groups |
|--------------------------------------|-------|-------|-------|
| Group 1 ($n = 7$)                   | Body weight (g) | MAP (mm Hg) | UprV ($\mu g/min$) | GFR (ml/min) | RPF (nl/min/mm Hg) |
| Normal control                      | 259   | 108   | 6     | 0.97   | 3.6           |
| Group 2 ($n = 12$)                  | 267   | 105   | 65*   | 1.05   | 4.3           |
| (PHN, vehicle)                      | 8     | 7     | 8     | 0.10   | 0.3           |
| Group 3 ($n = 6$)                   | 273   | 91†   | 38†   | 0.90   | 5.1†          |
| (PHN, acetylcholine)                | 9     | 3     | 6     | 0.10   | 0.2           |
| Group 4 ($n = 8$)                   | 262   | 126†  | 123†  | 0.92   | 2.3†          |
| (PHN, angiotensin II)               | 10    | 5     | 24    | 0.07   | 0.2           |

PHN, passive Heymann nephritis; MAP, mean systemic arterial pressure; UprV, total kidney protein excretion rate; GFR, whole kidney glomerular filtration rate; RPF, whole kidney effective plasma flow rate.

Table 2. Summary of Various Glomerular Microcirculatory Parameters in Four Experimental Groups

<table>
<thead>
<tr>
<th>SNGFR (nl/min)</th>
<th>$P_{oc}$ (mm Hg)</th>
<th>$\Delta P$ (mm Hg)</th>
<th>$C_A$ (g/dl)</th>
<th>$Q_A$ (nl/min)</th>
<th>$R_A$ (mm Hg/(nl/min))</th>
<th>$R_E$ (nl/min/mm Hg)</th>
<th>$K_r$ (nl/min/mm Hg)</th>
<th>SNFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 ($n = 7$)</td>
<td>43.7</td>
<td>49</td>
<td>33</td>
<td>5.7</td>
<td>146</td>
<td>0.25</td>
<td>0.15</td>
<td>6.30</td>
</tr>
<tr>
<td>Normal control</td>
<td>4.7</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
<td>18</td>
<td>0.02</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Group 2 ($n = 12$)</td>
<td>39.8</td>
<td>55*</td>
<td>40*</td>
<td>5.5</td>
<td>123</td>
<td>0.26</td>
<td>0.23*</td>
<td>2.88*</td>
</tr>
<tr>
<td>(PHN, vehicle)</td>
<td>2.3</td>
<td>1</td>
<td>1</td>
<td>0.3</td>
<td>9</td>
<td>0.03</td>
<td>0.02</td>
<td>0.36</td>
</tr>
<tr>
<td>Group 3 ($n = 6$)</td>
<td>35.8</td>
<td>50†</td>
<td>35†</td>
<td>5.4</td>
<td>143</td>
<td>0.19</td>
<td>0.14†</td>
<td>3.00</td>
</tr>
<tr>
<td>(PHN, acetylcholine)</td>
<td>2.5</td>
<td>2</td>
<td>1</td>
<td>0.2</td>
<td>17</td>
<td>0.03</td>
<td>0.02</td>
<td>0.48</td>
</tr>
<tr>
<td>Group 4 ($n = 8$)</td>
<td>37.4</td>
<td>67†</td>
<td>52†</td>
<td>5.5</td>
<td>96</td>
<td>0.40†</td>
<td>0.41†</td>
<td>1.80†</td>
</tr>
<tr>
<td>(PHN, angiotensin II)</td>
<td>3.7</td>
<td>2</td>
<td>2</td>
<td>0.1</td>
<td>13</td>
<td>0.06</td>
<td>0.05</td>
<td>0.18</td>
</tr>
</tbody>
</table>

PHN, passive Heymann nephritis; SNGFR, single nephron glomerular filtration rate; $P_{oc}$, glomerular capillary hydraulic pressure; $\Delta P$, glomerular transcapillary hydraulic pressure difference; $C_A$, afferent arteriolar plasma protein concentration; $Q_A$, initial glomerular plasma flow rate; $R_A$, afferent arteriolar resistance; $R_E$, efferent arteriolar resistance; $K_r$, ultrafiltration coefficient; SNFF, single nephron filtration fraction. Efferent arteriolar colloid osmotic pressure-to-glomerular transcapillary hydraulic pressure difference ratio was found to be less than 1.0 in 4 Group 1 rats, 11 Group 2 rats, 4 Group 3 rats, and 8 Group 4 rats. In these animals, unique $K_r$ values were calculated and pooled with minimum $K_r$ values to obtain average values.

Values show statistically significant ($p<0.05$) difference from those of Group 1 (*) or Group 2 (†) by simultaneous multiple comparisons of the Bonferroni method following one-way ANOVA. Values are expressed as mean $\pm$ SEM.
Proportionally, this tendency was more marked in R_E than R_N (only R_E reached statistical significance), accounting for the observed high plasma flow rate and depressed P_{OC} in Group 3 rats. Finally, these tendencies in glomerular microcirculatory patterns were associated with a significantly lower level of UprV in Group 3 animals, treated with acetylcholine, when compared with those measured in Group 2 nontreated rats (Table 1).

In Group 4 animals given angiotensin II, most of the patterns described above for Group 3 were reversed. Thus, while values for total GFR and SNGFR remained at levels comparable to those of Group 2, MAP, P_{OC}, and ΔP were significantly and markedly elevated over the corresponding values of Group 2 (Tables 1 and 2). Also, values for both RPF and Q_L tended to be substantially lower than those of Group 2 nontreated rats. These patterns in glomerular pressures and flows resulted from a greater degree of increase in R_E than R_N from corresponding values of Group 2, the average R_E value reaching that of R_N in Group 4 animals. During angiotensin II infusion, values for K_f further decreased in rats with PHN. Thus, K_f values averaged 1.80 ± 0.18 nl/(min/mm Hg) in Group 4, a value significantly lower than that of Group 2. The above described glomerular microcirculatory patterns in Group 4, which were directionally opposite to those seen in Group 3, were also associated with an accentuation of the pattern of uric acid excretion. Thus, Group 4 rats had UprV values significantly higher than those measured in Group 2 (Table 1).

Dextran Clearance Measurements

The results of fractional dextran clearance measurements in animals from Groups 1–4 are summarized in Figure 1. The fractional clearance of dextran, θ, indicates the glomerular transcapillary transport rate of any given size of dextran molecule relative to that of inulin, which is freely permeable across the capillary wall. As shown, for Group 1 normal control rats, this index decreased progressively as the size of dextran molecules increases, reflecting the size-selective barrier function of the glomerular capillary wall. Group 2 nontreated rats with PHN were characterized by significantly (p<0.05) enhanced θ for molecular radii greater than 50 Å when compared with Group 1 normal control rats. Administration of acetylcholine, which depressed ΔP and caused diminution of UprV in Group 3 rats, was also shown to bring about a shift of θ values for higher molecular size dextrans toward the values observed in normal animals (Group 1) [molecular radii greater than 40 Å were significantly (p<0.05) higher than in Group 1 (control)] normal controls]. In Group 3 (PHN+Ach) (PHN rats, acetylcholine-treated), θ values for molecular radii greater than 40 Å were significantly lower (p<0.05) than in Group 2. However, these values in Group 3 were still higher than those found in Group 1. In Group 4 (PHN+AII) (PHN rats, angiotensin II-treated), θ values for molecular radii greater than 26 Å were significantly (p<0.05) higher than those in Group 2.

Calculation of Membrane Parameters

Employing the mathematical model of Deen et al., membrane parameters that determine the macromolecular fluxes were calculated for all four experimental groups. The results are shown in Figures 2A and 2B.
As shown in Figure 2A, calculated values for $r_0$, the radius of small selective pores composing the bulk of the membrane, were similar in all groups (43.8, 46.5, 44.1, and 48.9 Å in Groups 1–4, respectively). By contrast, a marked difference among the groups was noted in the value for $\omega_0$, the parameter related to the fraction of GFR passing through large, nonselective pores. As shown in Figure 2B, $\omega_0$ was 0.74 x 10$^{-4}$ in Group 1 normal control and 3.98 x 10$^{-4}$ in Group 2 nontreated PHN rats. Of note, substantial decrease and increase in this parameter were seen during acetylcholine-induced reduction and angiotensin II-induced elevation in $\Delta P$, respectively. The values of $\omega_0$ were 2.89 x 10$^{-4}$ for Group 3 and 10.9 x 10$^{-4}$ for Group 4.

**Discussion**

We have demonstrated previously in a rat model of renal vein obstruction that acute upward or downward changes in $\Delta P$ induced parallel changes in the urine protein excretion rate. In that study, $\Delta P$ was acutely elevated in normal Munich-Wistar rats by partial occlusion of the renal vein and subsequently returned to near baseline levels by infusion of the angiotensin II antagonist saralasin while renal vein constriction was maintained. Moreover, changes in urinary protein excretion rate were also accompanied by parallel changes in fractional clearances of large dextrans. In preliminary studies in PHN, we have also attempted to inhibit the effect of endogenous angiotensin II using specific pharmacologic blockades, i.e., saralasin (0.3 mg/kg/hr i.v., n = 2 rats) or teprotide (6 mg/kg/hr i.v., n = 2 rats). The results were unrevealing and therefore are not presented; these treatments failed to change $\Delta P$ or UprV to any appreciable extent. Infusion of OKY-1581 (40 mg/kg/hr i.v., n = 2 rats), a thromboxane synthesis inhibitor, was likewise without effect on $\Delta P$ or proteinuria. Since our attempts to seek a specific biologic substance responsible for the proteinuria failed, we redirected our approach to determine the role played by $\Delta P$ on the degree of proteinuria in this form of glomerular immune injury. In designing the protocol, it was felt that our experimental goal could best be accomplished by manipulating $\Delta P$ with the use of pharmacologic agents that exert their actions through different mechanisms.

The decrement in $\Delta P$ attained by acetylcholine infusion from the abnormally high levels of Group 2 toward normal control levels of Group 3 was associated with a simultaneous decrease in urinary protein excretion and of larger dextran molecules (Tables 1, 2, and Figure 1). In contrast, further increment in $\Delta P$ induced by angiotensin II (Group 4) led to concurrently greater increases in protein excretion and fractional clearances of larger dextrans (Tables 1, 2, and Figure 1). This pattern of changes essentially duplicates those observed during renal vein constriction.

To determine whether the close association observed between $\Delta P$ and macromolecule filtration was simply the result of effects on the convective and diffusive driving forces, or of alterations in actual barrier properties, we used a mathematical model to calculate membrane parameters from the clearance and micropuncture data. According to this model, which has been successful in correlating dextran sieving data in several forms of glomerular injury, the glomerular size selectivity is characterized by two parameters, $r_0$ and $\omega_0$. Our calculations revealed that during each of our three experimental manipulations (induction of PHN, and infusion of acetylcholine or angiotensin II), changes in $\Delta P$ were accompanied by parallel changes in the fraction of filtrate passing through large, nonselective pores (related to $\omega_0$), while the pore radius of the predominant pathway for water ($r_0$) remained essentially unchanged. The calculated changes in $\omega_0$ indicate that the observed variations in $\Delta P$ and in the other determinants of SNGFR would be inadequate to explain the dextran sieving data if no structural alteration in the glomerular barrier were postulated.

In Groups 2 and 4, changes in $\Delta P$ were accompanied by alterations in another membrane parameter, the ultrafiltration coefficient ($K_1$). In those groups, values for $K_1$ changed in a direction opposite to that of $\Delta P$ and, hence, opposite to $\omega_0$ as well. Assuming that almost all water movement is through the small pores, $K_1$ is defined as

$$K_1 = \frac{fS}{\eta}$$

where $f$ denotes the fraction of capillary surface area occupied by small pores, $S$ the total glomerular capillary surface area, $l$ the small pore length, $r_0$ the pore radius of small pores, and $\eta$ the viscosity of the glomerular filtrate. Since the value of $r_0$ in PHN rats remained essentially unchanged compared to normal control rats (Figure 2A), decrease in $K_1$ in the PHN rats must be attributed to a low level of total small pore area ($fS$), or increased pore length ($l$), or a combination thereof. While a thickening of the glomerular basement membrane is a constant finding in patients with membranous glomerulopathy, a factor that could account for a reduced $K_1$ by virtue of the increased pore length, it is unlikely that this phenomenon played a crucial role in the observed reduction in $K_1$ in this acute model of PHN studied by us only 10 days following the administration of the heterologous antibodies. Our findings, therefore, suggest a reduction in $fS$ as the cause of the observed reduction in the calculated value of $K_1$.

The possible changes in capillary wall in PHN are shown schematically in Figure 3. The reduction in $K_1$ may have resulted from a decrease in $f$ (the fraction of surface area occupied by small pores, shown at lower left) and/or $S$ (the total surface area, shown at lower right). In either case, the increased $\omega_0$ implies an increased ratio in the number of large pores versus small pores. Note that the increase in the relative
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Mathematical interpretation of changes occurring in the channels for water and macromolecular transport in the glomerular capillary wall of early passive Heymann nephritis (PHN). Top diagram shows normal condition of glomerular capillary membrane. Small numbers of large nonselective pores are distributed among large numbers of small size-selective pores. In PHN rats (lower diagrams), an increase in large-to-small pore population ratio, which accounts for the observed increase in fractional volume flux passing through nonselective pathways, is associated with either a decrease in the density of small selective pores (bottom left) and/or a decrease in the total surface area (bottom right), both of which effectively decrease ultrafiltration coefficient, $K_f$.

A close association between $\Delta P$ and $\omega_o$, as was observed in the present study, has also been reported previously in other experimental manipulations. Thus, it is speculated that $\Delta P$ would play a major role in determining the magnitude of proteinuria and changes in $\omega_o$ by new recruitment of previously unexposed nonselective pathways. However, remaining undetermined is the extent to which the inferred increases in $\omega_o$ are exclusively the result of elevations in $\Delta P$ in PHN. The lowering of $\Delta P$ during acetylcholine infusion reduced $\omega_o$ toward the normal control value, but we were not able to reduce $\Delta P$ strictly to the baseline level. On the other hand, it has been clearly demonstrated that proteinuria in PHN is complement mediated. There is, therefore, a potential structural lesion that may account for an elevated basal level of $\omega_o$, which may be further aggravated by a rise in $\Delta P$.

In this regard, increased $\omega_o$ and reduced $f$ and/or $S$ found in our model of early membranous glomerulopathy is in keeping with the previously observed alteration of glomerular epithelial cell architecture in this model that leads to flattening, simplification, and retraction of foot processes and a reduction in the number of filtration slits available for filtration of water and electrolytes. At the same time, the formation of immune complexes on or near the podocyte cell membrane initiates the activation of the complement cascade that culminates in the generation of the membrane attack complex. The ensuing local damage to epithelial cells results not only in distortion of the filtration slits, as observed in the experimental animal, but also may cause severe damage and necrosis of epithelial cells, with detachment of podocytes from the underlying basement membrane as demonstrated in an isolated kidney perfusion system. Such local distortion of the epithelial cell layer with denudation of the basement membrane on the urinary side may represent an exaggerated structural equivalent of the large, nonselective pores, a pathway permeable to macromolecules. In any case, the magnitude of this nonselective pathway seems highly dependent on $\Delta P$ as suggested by the results obtained in the present set of experimental manipulations using vasoactive substances.

Acknowledgment

The authors express their thanks to Ms. Laurette Hughes for technical assistance.

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**Key Words** • glomerular permselectivity • proteinuria • micropuncture • transcapillary hydraulic pressure difference • Heymann nephritis
Role of abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: a study in early passive Heymann nephritis.
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Circ Res. 1987;61:531-538
doi: 10.1161/01.RES.61.4.531

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