Ultrafiltration coefficient and glomerular capillary resistance in a model of immune complex glomerulonephritis

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Ultrafiltration coefficient and glomerular capillary resistance in a model of immune complex glomerulonephritis. Decreased ultrafiltration coefficient, L_pA or K_f, was documented previously in micropuncture studies of glomerulonephritis in rats. Observations were made immediately following an injection of antiglomerular basement membrane (anti-GBM) antibody, later in the course of glomerulonephritis, and during the chronic phase of Heymann nephritis. To gain further insight into the basis of reduced glomerular filtration rate in immune-complex glomerulonephritis, we studied the anatomic, physiologic, and rheologic properties of isolated glomeruli from female Buffalo rats with nephritis which developed during infection with *Trypanosoma rhodesiense*. Immune-complex mediated glomerulonephritis was present 2 weeks after inoculation and progressed throughout the 4 weeks of study. Renal insufficiency occurred, with serum creatinine concentrations rising to 5 to 10 times control values by week 4. Mesangial hypercellularity, mesangial electron dense deposits, and endothelial cell swelling were observed. Increased numbers of mononuclear cells were present within the glomerulus. Total glomerular water volume was greater in nephritic than in normal animals. Increased cell volume accounted for most of the volume increment. When filtration into the capillaries was induced in vitro by imposing an oncotic gradient of 6.5 mm Hg or greater across the capillary wall, rapid and uniform erythrocyte movement occurred within the capillaries of control glomeruli and erythrocytes were ejected into the medium. In contrast, a transcapillary gradient of 30 to 40 mm Hg was required to produce erythrocyte movement in glomeruli from nephritic animals studied 4 weeks after inoculation. The ultrafiltration coefficient of nephritic glomeruli was estimated in vitro and was not different from that of control glomeruli (5.81 ± 0.35 vs. 6.21 ± 0.49 nl/min mm Hg). An impairment of capillary perfusion may be responsible for the decreased rate of glomerular filtration observed in this model of glomerulonephritis.

Evaluation in vitro du coefficient d'ultrafiltration et de la résistance capillaire glomérulaire dans un modèle de glomérulonéphrite des comples immuns. La diminution du coefficient d'ultrafiltration, L_pA ou K_f, a été établie précédement au cours de travaux utilisant les microponctions chez des rats atteints de glomérulonéphrite, immédiatement après l'injection d'anti-corps anti-membrane basale glomérulaire (anti-GBM) et, ultérieurement, au cours de l'évolution de glomérulonéphrite et durant la phase chronique de la néphrite de Heymann. Afin d'obtenir plus d'informations sur les fondements de la diminution du débit de filtration glomérulaire au cours de la néphrite des complexes immuns, nous avons étudié les propriétés anatomiques, physiologiques, et biologiques des glomérules isolés de rats femelles de la souche Buffalo atteints de néphrite développée au cours de l'infection par *Trypanosoma rhodesiense*. Une glomérulonéphrite des complexes immuns

existait deux semaines après l'inoculation et évoluait pendant les 4 semaines de l'étude. Il existait une insuffisance rénale et la créatinine sérique atteignait des valeurs 5 à 10 fois plus grandes que les contrôles à la 4 semaine. L'hypercellularité mésangiale, sous la forme de dépôts denses mésangiaux en microscopie électronique, et le gonflement des cellules endothéliales ont été observés. Le nombre des cellules mononucléés du glomérule était augmenté. Le volume total d'eau du glomérule était plus grand chez les animaux atteints de néphrite que chez les contrôles. L'augmentation du volume cellulaire rendait compte de la plus grande partie de l'augmentation de volume. Quand la filtration dans les capillaires a été declenchée par l'imposition d'un gradient oncotique de 6,5 mm Hg ou plus à travers la paroi capillaire, un mouvement rapide et uniforme des érythrocytes est apparu et les érythrocytes ont été éjectés dans le milieu. Par contre, pour les glomérules provenant d'animaux néphritiques, étudiés quatre semaines après l'inoculation, un gradient de 30 à 40 mm Hg était nécessaire pour produire un mouvement des érythrocytes. Le coefficient d'ultrafiltration des glomérules d'animaux néphritiques a été évalué in vitro et n'est pas différent de celui des animaux contrôles (5,81 ± 0,35 vs. 6,21 ± 0,49 nl/min mm Hg). L'altération de la perfusion capillaire est responsable de la diminution du débit de filtration glomérulaire observée dans ce modèle de glomérulonéphrite.

The contrast between the severity of histologic glomerular involvement and the decrease in glomerular filtration in glomerulonephritis in man presents a difficult conceptual problem to clinicians and pathologists alike. In some cases, glomerular filtration remains normal although glomeruli are severely altered by inflammation or scarring; in others renal failure may occur with mild glomerular alterations. This dissociation has been illustrated by the finding that glomerular filtration rate is more closely correlated to tubulointerstitial than to glomerular morphologic alterations even in primary glomerular diseases [1]. Recent micropuncture studies in experimental glomerulonephritis in rats have documented decreased ultrafiltration coefficient (K_f or L_pA) and have revealed the importance of compensatory alterations in renal plasma flow and glomerular afferent arteriolar resistance in maintaining glomerular filtration in damaged glomeruli [2, 3, 4]. Conversely, the ability to autoregulate filtration rate when cardiac output or arterial pressure is decreased may be limited in glomerulonephritis because maximal afferent arteriolar dilatation and increased glomerular perfusion may already be present [5].

In our study, we applied a new technique for studying glomerular filtration in vitro [6] to glomeruli from Buffalo rats infected with *Trypanosoma rhodesiense*. We chose to study this model because the glomerulonephritis was diffuse, affecting all glomeruli to a similar degree. It occurred in conjunction with

circulating immune complexes produced in response to infection, and increasing histologic involvement was associated with progressive renal insufficiency. In addition, the immunologic features of this syndrome in the rat have been well characterized previously [7–8]. Of the several strains of rats we studied, the Buffalo rat developed the most severe, predictable, and generalized glomerular involvement [7]. Because the Buffalo rat does not have surface glomeruli which can be punctured directly, we studied filtration by isolated glomeruli in vitro. Filtration was produced by creating an oncotic gradient across the glomerular capillary. The resulting filtration was documented by an increase in capillary size and by erythrocyte ejection that began after approximately 0.2 sec and persisted up to 60 sec. The ultrafiltration coefficient (K_f) was estimated from the initial rate of volume change and the oncotic gradient applied for each glomerulus studied. Isolated glomeruli from nephritic rats had a normal K_f, but erythrocyte movement along the glomerular capillaries was markedly impaired. We suggest that altered glomerular perfusion, at least in part due to segmentally increased intraglomerular resistance, may account for the decreased glomerular filtration in this model of glomerulonephritis.

Methods

Female Buffalo rats weighing 200 to 250 g (Simoneson Laboratories, Inc., Gilroy, California, USA) were infected by an i.p. injection with 1000 organisms of *Trypanosoma rhodesiense* (EATRO 1886, Walter Reed Army Institute of Research, Washington, D.C., USA) on day zero as previously reported [7]. Infection was confirmed by noting the presence of trypanosomes in blood obtained at the time the rats were killed. Three groups of 8 to 15 rats weighing 150 to 200 g, inoculated during a one-year period, were studied. Rats were studied from 1 to 4 weeks after inoculation. Control rats were studied at the same intervals. All rats were allowed free access to water and standard laboratory food for the duration of the study. In some animals, urine samples for clearance measurements were collected overnight in metabolic cages prior to sacrifice. Serum samples for the determination of creatinine and for immunologic studies were obtained at the time of sacrifice. Urine and serum creatinine samples were measured by an automated analytic method, and total serum protein was estimated by refractometry (Total Solids Meter, American Optical, St. Louis, Missouri, USA). Renal clearances were calculated in the standard manner.

Isolation of glomeruli. Rats were anesthetized with ether and killed by exsanguination. Kidneys were removed promptly via a midline abdominal incision and placed in media at room temperature. The medium for all experiments consisted of isotonic salt solution which contained, in millimoles per liter: sodium chloride 115, potassium chloride 5, sodium acetate 10, sodium phosphate 1.2, sodium bicarbonate 25, magnesium sulfate 1.2, calcium chloride 1.0, and glucose 5.5. All experiments were performed at room temperature. The pH of the bathing medium was adjusted to 7.4 by equilibration with 5% carbon dioxide and 95% oxygen immediately prior to usage. Bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Missouri) was added to the medium to give final concentrations of 3 or 4 g/dl. The concentration of BSA was estimated by refractive index (Total Solids Meter, American Optical, St. Louis, Missouri). A sec-

tion of kidney from each animal was fixed in cold 1% glutaraldehyde for light and electron microscopy.

Glomeruli were isolated from the renal cortex as previously reported [6]. Briefly, the renal cortex was removed and minced into 1 to 2 mm fragments with stainless steel scissors. Fragments were than pressed through 80 mesh stainless steel screens. Glomeruli and some tubular fragments passed through these screens and were suspended in the media. Glomeruli for permeability studies were selected directly from this preparation. Glomerular suspensions for cell volume measurement were further purified by passing them through sieves of finer mesh. Pure glomeruli were most often recovered from above a 200 mesh screen. When a medium containing 3 or 4 g/dl BSA was used, erythrocytes were retained within the glomerular capillaries.

Glomerular permeability studies. Glomerular filtration was induced in isolated glomeruli by applying an oncotic gradient across the glomerular wall [6]. An intact glomerulus was selected and held on a micropipette by continuous suction. The glomerulus was observed at ×400 using a Leitz Epivert inverted microscope, and the video image was recorded using a video camera (HB, 40S Hitachi Electronics, Ltd., Japan) and a Sony (RC–505) videocorder equipped with VMI RC–505 stop-action control (Video Masters Inc., Kansas City, Missouri). Serial observations and measurements of glomerular size were obtained from the video image at 1/60-sec intervals. The magnification factor for these studies was ×1333. Glomerular volume was estimated from the average of 4 measured diameters by the formula $V = 4/3 \pi r^3$.

For the studies described, glomeruli were isolated in a medium containing 3 or 4 g/dl BSA. Glomeruli were incubated in this medium at room temperature until they were selected for study 15 to 60 min after the beginning of the isolation procedure. During glomerular isolation and incubation the osmotic and oncotic components of the fluid within the capillaires equilibrated with the bathing medium because the glomerular capillary is very permeable to water and small solutes and would not maintain a significant gradient for the long periods involved. After selection each glomerulus was washed with medium containing 1 or 2 g/dl BSA. Oncotic pressure of media with these protein concentrations was calculated using the Landis-Pappenheimer equation [9]. These values agreed well with those measured by membrane oncometer (Wescor Inc., Logan, Utah). The abrupt media change to 1 or 2 g/dl BSA created an oncotic gradient of 12 or 8.5 mm Hg, respectively, across the capillary wall. This gradient acted as a driving force for net filtration into the glomerular capillaries. Several events occurred as soon as the new medium reached the glomerulus. First, the glomerular size increased abruptly. This size change continued for about 0.2 sec. The maximum rate of volume change occurred within 1 to 4 1/60-sec intervals. The glomerular volume stabilized after about 0.2 sec and further filtration caused the visible linear movement of erythrocytes along the glomerular capillary and the ejection of erythrocytes (and plasma) from fragments of arterioles at the vascular pole. Ejection continued for up to 60 sec after media change in control glomeruli. Ejection was impaired in some glomeruli from nephritic rats (vide infra), but glomerular volume increase followed a similar time course to that of glomeruli from control rats. K_f was calculated from the initial rate of change of

glomerular volume in response to the oncotic gradient using the equation:

$$K_f = \Delta v / \Delta t \cdot \Delta \pi$$

where Δv is largest volume increment in the first 0.1 sec after the medium changes, Δt is 1/60 sec, and $\Delta \pi$ is the difference between the oncotic pressures of the isolation and washing media.

Estimation of glomerular volume by radioisotope methods. Total glomerular volume and cell volume per glomerulus were estimated from the ^3H_2O and ^{14}C-inulin spaces of glomeruli [6]. Briefly, suspensions containing approximately 10^4 glomeruli/ml of medium were incubated for 30 to 60 min at 25° C in isotonic medium containing 1 g/dl BSA and ^3H_2O and ^{14}C-inulin. A medium containing 1 g/dl BSA was chosen for these incubations and was identical to the final medium in the filtration experiments described below. After this period, 100 μl aliquots of the suspension were centrifuged through 500 μl of silicon oil (Contour Chemical Corp., Woburn, Massachusetts) in Eppendorf tubes using a Beckman microfuge (Model B, Beckman, Palo Alto, California). Glomeruli, recovered by freezing the tubes in liquid nitrogen and cutting off the tips, were digested in 5% trichloroacetic acid (TCA). The resulting suspension was mixed with scintillation cocktail (Aquasol⑬, New England Nuclear, Boston, Massachusetts) and counted in a liquid scintillation counter (Searle Analytic, Mark III 6880, Des Plains, Illinois). A sample of supernatant was also digested with TCA and mixed with scintillation fluid and counted in an identical manner and CPM as determined using the differential energies of ^{14}C and ^3H. ^3H_2O or ^{14}C-inulin space per glomerulus was calculated from the formula:

$$\frac{\text{glomeruli total dpm}}{\text{medium dpm/μl} \times \text{total number of glomeruli}}$$

The total number of glomeruli in an aliquot was determined by counting glomeruli in replicate aliquots using the inverted microscope. ^3H_2O space was interpreted as an approximation of total glomerular water volume and ^3H_2O space minus ^{14}C-inulin space was taken as intracellular water.

Qualitative estimates of glomerular rheologic properties. The rheologic properties of the glomerular capillary were assessed qualitatively each time a filtration experiment was performed as glomeruli isolated in 4 g/dl BSA were washed with 1 of 2 g/dl BSA. The rate of erythrocyte movement was graded from 0 to 4+. Zero denoted no movement; 1+, minimal movement of a few cells; 2+, interrupted or segmental movement; 3+, sustained movement with little impairment of flow; and 4+, vigorous movement throughout the glomerulus. The presence or absence of deformation of erythrocytes during passage through the capillaries and of intermittent cessation of movement were also noted. From these observations we attempted to determine whether interference with erythrocyte movement was caused by obstruction within the capillary lumens or at the sites of ejection.

We also examined the effect of a more rapid influx of medium into the capillary lumens by using differences of up to 40 mm Hg between the oncotic pressures of the initial and final media. For these studies, we isolated glomeruli from rats 3 to 4 weeks after inoculation in media with BSA concentrations of 6 and 8 g/dl. Gradients of 40 (8 to 0 g/dl BSA), 30 (8 to 3 g/dl BSA), and 26

mm Hg (8 to 4 or 6 to 0 g/dl BSA) were used to produce filtration.

Immune complex detection. Immune complexes were measured by polyethylene glycol precipitation [10]. In brief, 0.16 ml of rat serum was precipitated with polyethylene glycol (final concentration 3.5%); the precipitate was washed once, dissolved in 5 ml 0.1 N sodium hydroxide and the absorbance at 280 nm measured.

Detection of trypanosomal antibodies. IgG antibodies to trypanosomes were measured with an enzyme immunoassay [8] as modified from previously published methods [11, 12]. Cuvettes were sensitized with soluble trypanosomal antigens or with BSA as the control. Each rat serum sample was tested at 1:100 dilution. Determinations were carried out in triplicate and the mean absorbance (2 min following substrate addition) at 405 nm reported.

Light and electron microscopy. Tissues were prepared for light and electron microscopy as previously described [7]. Portions of one kidney from each rat were fixed in 2% buffered glutaraldehyde. Light microscopy was performed on fixed tissues which had been dehydrated in graded alcohols, embedded in paraffin, cut in sections 2 to 3m thick and stained with hematoxalin and eosin or periodic acid schiff reagent. Additional portions of fixed tissue were embedded in Spurs epoxy resin for electron microscopy. Thin sections were stained with lead citrate and uranyl nitrate and examined in a Hitachi 12 transmission electron microscope.

Statistical Analysis. Group mean values were compared by the Student's *t* test for unpaired observations. Erythrocyte movement within glomeruli was compared using the chi-square analysis.

Results

Clinical course of infection. All rats demonstrated parasitemia by 1 week after inoculation. Parasitemia increased during the course of the infection. Splenomegaly was a consistent finding by 2 weeks, and ascites were present by 4 weeks. Hypoproteinemia and visible lipemia were found at 3 to 4 weeks. Serum creatinine concentration was increased over control values in each animal sacrificed at 2 weeks or after and continued to rise throughout the study as shown in Table 1. Creatinine clearance was decreased in each of the eight nephritic animals in which it was measured. Anti-trypanosomal antibodies and elevated levels of circulating immune complexes were present at 2 weeks and persisted through the study (Table 1).

Histologic studies. Examination of glomeruli by light microscopy revealed diffuse glomerulonephritis in every infected rat. Mesangial hypercellularity was observed as early as 2 weeks after inoculation and increased during the 4 weeks of the study. Endothelial cells were prominent and many capillary lumens were apparently narrowed by 4 weeks. Few polymorphonuclear leukocytes were observed but numerous mononuclear cells were present within capillaries. Mild interstitial edema and focal lymphocytic interstitial infiltration were present. By 3 weeks, proximal tubular cells contained numerous PAS positive hyaline droplets.

Transmission electron microscopy of infected rat glomeruli revealed endothelial damage characterized by cellular swelling and focal loss of endothelial fenestrae. The capillary lumens appeared segmentally collapsed with convolution and apparent

Table 1. Clinical course of trypanosomal glomerulonephritis[a]

	S_{Cr}		C_{Cr}		Serum protein		Serum turbity	Immune complexes		Anti tryp ab	
	mg/dl	N^b	*ml/min*	N	*mg/dl*	N		*A-280*	N	*net A 405*	N
Control	0.33 ± 0.06	11	0.48 ± 0.10	5	7.0 ± 0.8	11	0/10	0.02 ± 0.01	4	0.10 ± 0.03	4
14 days	0.81 ± 0.40	7	0.26 ± 0.11	4	7.5 ± 0.1	2	3/7	0.25 ± 0.16	2	0.55 ± 0.10	2
21 days	1.06 ± 0.48	7	0.24 ± 0.08	4	5.8 ± 0.9	4	6/8	0.14 ± 0.05	2	0.26 ± 0.04	2
28 days	1.62 ± 0.87	6	not done		5.3 ± 0.7	5	3/3	0.30 ± 0.21	3	0.29 ± 0.09	4

[a] Values are mean ± SEM.
[b] N represents number of animals.
Abbreviations: S_{Cr}, serum creatinine concentration; C_{Cr}, creatinine clearance.

thickening of basement membranes. In many of the capillaries red blood cells appeared compressed into narrowed lumens. Occasional trypanosomes and activated monocytes were also seen within the lumens. The mesangial matrix appeared expanded in volume and contained numerous mesangial cell cytoplasmic processes. Figure 1 reveals electron dense deposits which were commonly seen in the mesangial regions but were not seen in capillary walls.

Glomerular volume estimates by radioisotope spaces. Total water volume per glomerulus, as estimated from the ^3H_2O space was greater in nephritic than in control glomeruli. Most of the increment in total volume was accounted for by intracellular volume, estimated as ^3H_2O space minus ^{14}C-inulin space (Table 2).

Demonstration of glomerular filtration in isolated glomeruli, estimation of ultrafiltration coefficient. The initial geometric volume of the glomeruli in which filtration was studied did not differ between control and nephritic glomeruli. Every glomerulus studied increased in size when the medium was changed from 4 to 1 or 2 g/dl BSA. This increase was complete within about the first 0.2 sec after the initiation of the medium change in both control and nephritic glomeruli. Erythrocyte ejection, when present (vide infra), occurred following the increase in glomerular size and persisted for up to 60 sec. The magnitude of the volume increase was the same in both groups, averaging about 0.04 nl. These observations indicated that filtration occurred in both control and nephritic glomeruli and suggested that glomerular compliance, reflected by the maximal increments in volume, was similar in both groups. Table 3 depicts that K_f for nephritic glomeruli from rats studied 2, 3, and 4 weeks after infection was not different from control values.

Resistance to erythrocyte flow within glomerular capillaries. When glomerular filtration was produced in control glomeruli, a calculated transcapillary oncotic gradient of 6.5 mm Hg or more produced brisk erythrocyte movement along the capillaries and vigorous ejection of erythrocytes from arteriolar remnants (Table 4). No obstruction to erythrocyte passage was evident either within the capillaries or at the ejection sites. Capillary lumens were large enough to permit erythrocytes to tumble freely as they were carried along. Under similar conditions, filtration occurred in nephritic glomeruli as noted above, but erythrocyte ejection was absent or impaired because narrowed capillary lumens blocked erythrocyte passage within segments of the glomerulus.

In glomeruli from kidneys 3 to 4 weeks after inoculation, gradients less than or equal to 26 mm Hg produced virtually no

cell movement. Those gradients in the range of 30 mm Hg produced some movement in most glomeruli, and a gradient of 40 mm Hg was required to produce vigorous movement (Table 4).

Discussion

In previous studies, we have demonstrated the development of diffuse proliferative glomerulonephritis in rats infected with *Trypanosoma rhodesiense* [7, 8]. This nephritis was characterized by hypercellularity and widening of the mesangium. Endothelial cell swelling was prominent. IgM, IgGl, IgGa, and IgA were present in a finely granular pattern in the glomerular capillary walls and mesangium. Mesangial electron dense deposits also were seen. Five strains of rats were studied. Of these, Buffalo rats developed the most consistent and severe glomerulonephritis. They had peak titers of IgM, IgGa, and IgGl antibodies to trypanosomal antigens at 10 days. Immune complexes were elevated at 10 through 30 days [8]. Studies of *T. rhodesiense* in rabbits have also demonstrated the development of proliferative glomerulonephritis [13]. In this species, monocytic infiltrates constitute a prominent component of the glomerular hypercellularity [14]. In the present experiments, Buffalo rats developed a generalized proliferative glomerulonephritis with increased glomerular cellularity and prominence of endothelial cells and apparent encroachment of endothelial and mononuclear cells into capillary lumens.

Measurements of fluid spaces were consistent with the morphologic appearance of control and nephritic glomeruli. The exchangeable water volume was increased in nephritic glomeruli compared to control glomeruli. This increase was principally due to increased intracellular (non-inulin space) water and was consistent with the histologic impression that there were increased numbers of intraglomerular cells and increased cell cytoplasm in diseased glomeruli. In both control and nephritic glomeruli, measurements of the fluid spaces also documented that the extracellular compartment, primarily intracapillary water, made up the major portion of glomerular water. The presence of a large volume of extracellular water is required for the calculation of K_f since we have assumed that changes in glomerular size during filtration are primarily due to changes in intracapillary (extracellular) volume.

Although the pathologic changes visible on light microscopy were relatively mild, kidney failure developed. Despite decreased creatinine clearance, filtration occurred when the glomeruli were washed with medium containing a lower protein concentration than that of the initial bathing medium. Both the initial rate of filtration and the subsequent increase in glomeru-
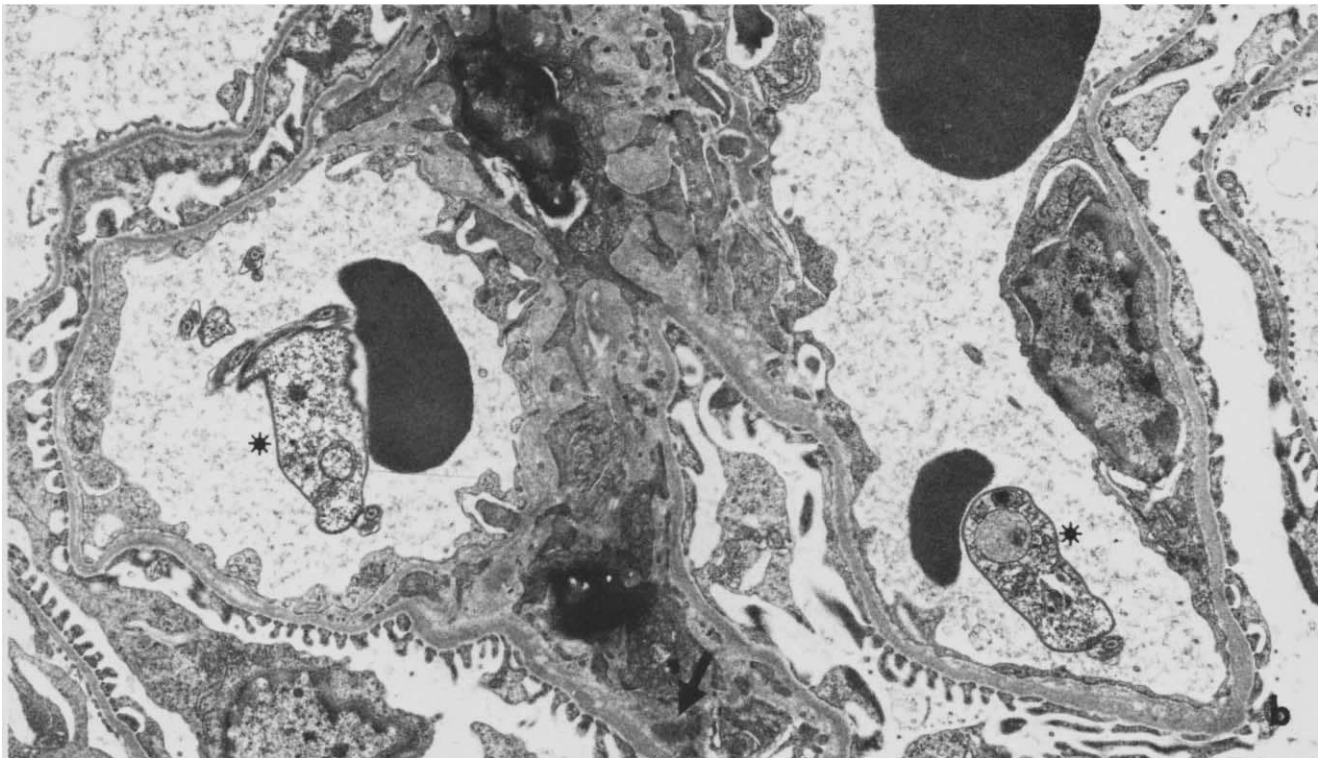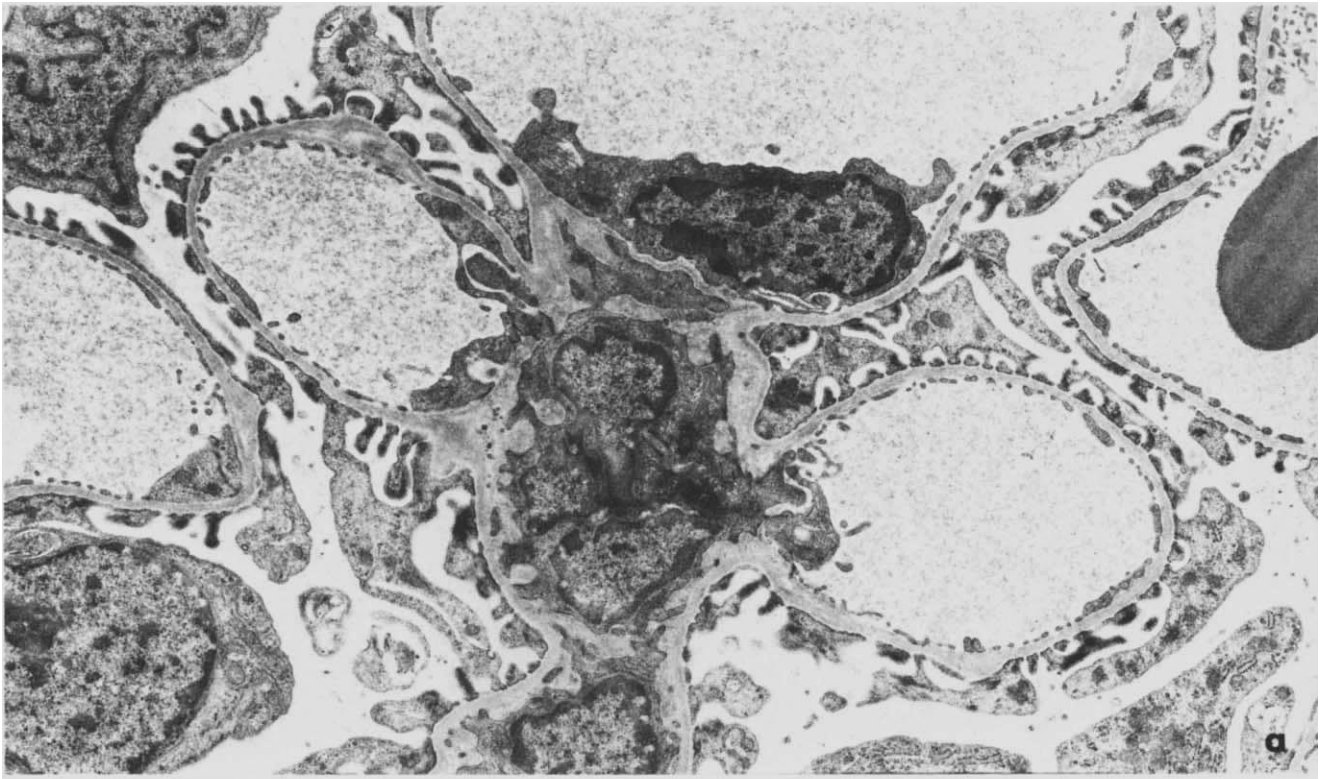
Fig. 1. a. *Electron micrograph of normal renal glomerulus from Buffalo strain rat.* Note fenestrations of normal endothelium and elaborate pedicle structure of the visceral epithelial cells. ×8300. **b.** *Electron micrograph of renal glomerulus in rat infected 21 days with Trypanosoma rhodesiense.* Note two Trypanosoma organisms (*) within the capillary lumen. The endothelial cells are swollen with generalized loss of fenestrae. The mesangial cells appear activated and rare, electron-dense deposits are seen in the perimesangial region (*arrow*). There is also local loss of visceral epithelial pedicles. ×8300.

Table 2. Radioisotope space of control and nephritic glomeruli[a]

	Total water (^3H_2O) *nl/glomerulus*	N[b]	Extracellular water (^{14}C-inulin) *nl/glomerulus*	N	Intracellular water (^3H_2O-^{14}C-inulin) *nl/glomerulus*	N
Control	1.14 ± 0.08	5	0.85 ± 0.06	5	0.30 ± 0.01	5
Nephritic	1.36 ± 0.07[c]	5	0.89 ± 0.05	5	0.47 ± 0.03[d]	5

[a] Values are mean $\pm$ SEM. All values were determined in suspensions of glomeruli incubated in isotopic medium containing 1 g/dl bovine serum albumin (BSA).
[b] N represents the number of animals.
[c] $P < 0.05$.
[d] $P < 0.001$.

Table 3. Geometric glomerular volume and ultrafiltration coefficient in control and nephritic rats[a]

	Initial volume[b] *nl/glomerulus*	N[c]	Final volume[d] *nl/glomerulus*	N	Final volume minus initial volume *nl/glomerulus*	N	K_f *nl/min mm Hg*[e]	N
Control	1.39 ± 0.06	10	1.43 ± 0.06	8	0.04 ± 0.002	8	6.21 ± 0.49	10
2 weeks	1.33 ± 0.06	3	1.40 ± 0.06	3	0.06 ± 0.005	3	6.29 ± 0.50	3
3 weeks	1.30 ± 0.11	3	1.37 ± 0.12	3	0.03 ± 0.005	3	5.49 ± 0.46	3
4 weeks	1.36 ± 0.05	5	1.40 ± 0.09	5	0.03 ± 0.002	5	5.72 ± 0.69	5

[a] Values are mean $\pm$ SEM.
[b] Initial glomerular volumes were determined during incubation in isotonic medium containing 3 or 4 g/dl bovine serum albumin (BSA).
[c] N represents the number of animals.
[d] Final glomerular volumes were determined after changing the medium to 1 gm/dl BSA. Final glomerular volumes in 2 animals were measured in 2 g/dl BSA and were omitted from these calculations.
[e] K_f was determined during change of medium from 3 or 4 g/dl BSA to 1 g/dl BSA medium; K_f represents the ultrafiltration coefficient.

Table 4. Erythrocyte movement in control and nephritic glomeruli caused by different transcapillary oncotic gradients

Transcapillary gradient	Erythrocyte movement		
	None	1 to 3+	4+
Control glomeruli[a]			
$\leq$ 26 mm Hg	0	5	51
Nephritic glomeruli[b]			
$\leq$ 26 mm Hg	27	4	0
30 mm Hg	2	15	0
40 mm Hg	0	1	16

[a] Control vs. nephritic glomeruli $\chi^2 = 54.3$, df $= 2$, $P < 0.0005$ gradient $\leq$ 26 mm Hg.
[b] Nephritic glomeruli — movement vs. gradient applied $\chi^2 = 91.5$, df $= 4$, $P < 0.0005$.

lar size caused by capillary distension during the inward filtration of fluid were equal in nephritic and control glomeruli. Calculated ultrafiltration coefficient was also normal. Striking differences in erythrocyte ejection from normal and nephritic glomeruli were observed during the measurement of glomerular filtration. When small gradients were used to produce filtration, erythrocytes were retained within the capillaries of nephritic glomeruli. When larger gradients were used, erythrocytes were ejected but they followed an interrupted and tortuous course within the capillaries. Erythrocytes moved freely within the capillaries of normal glomeruli and were vigorously ejected when filtration was produced by either small or large gradients. These observations suggested that there was increased resistance to erythrocyte ejection and that this resistance occurred within capillary lumens rather than at the arteriolar fragments at

the hilum. Increased capillary resistance may be secondary to intralumenal obstruction by enlarged endothelial cells, by marginated leukocytes, or by trypanosomes present within capillaries. Alternatively, the capillary may be compressed by mesangial edema or hypercellularity or by mesangial cell contraction.

We evaluated the extent of alteration in the rheological properties of the glomerular capillaries semi-quantitatively. We assumed that the minimum oncotic gradient required to cause erythrocyte movement reflected the intracapillary hydrostatic pressure required to overcome the forces which opposed erythrocyte ejection. Forces which might oppose erythrocyte ejection included the inertia of the erythrocytes, the friction between the erythrocytes and the capillary walls, the viscosity of the blood within the capillaries, and the resistance of the fragments of arterioles at the hilum. These forces cannot be measured individually in our in vitro experiments. Nevertheless, the minimum oncotic gradient required to produce any visible erythrocyte movement was estimated and was about 4 times greater in nephritic glomeruli than in control glomeruli. The minimum gradient required to produce vigorous movement and ejection was nearly five times greater in nephritic glomeruli.

Using micropuncture techniques, several investigators have previously studied the nature of the alterations in glomerular function which caused decreased glomerular filtration in glomerulonephritis. In these studies, factors which determine glomerular filtration rate including single nephron plasma flow, mean ultrafiltration pressure, and glomerular ultrafiltration coefficient were measured or estimated. Blantz, Tucker, and Wilson [4, 15] have studied glomerular dynamics within 60 min following the injection of antiglomerular basement membrane

antibody (AGBM Ab). During this period the single nephron filtration rate was markedly decreased. Afferent and efferent arteriolar resistances rose and renal plasma flow fell while the hydrostatic pressure gradient and mean effective filtration pressure increased. The effects of the increase in filtration pressure and the decrease in glomerular perfusion cancelled each other. Thus decreased filtration rate appeared to be caused by a decreased ultrafiltration coefficient (LpA). Because capillary loops were patent in this model, it seemed that a decrease in hydraulic conductivity (Lp) was probably responsible for the lowered ultrafiltration coefficient. Further studies in the same model [15] suggested that both the vasoconstriction and the decrease in LpA were related to the dose of AGBM Ab administered. In these studies complement depletion prevented migration of polymorphonuclear leukocytes into capillaries and eliminated the vasoconstriction. Complement depletion ameliorated but did not eliminate the effect of high dose AGBM Ab on LpA. We concluded that two processes were involved in lowering LpA in this model. The first was a complement dependent and related to loss of surface area (A) due to accumulation of polymorphonuclear leukocytes. Additionally, a complement independent endothelial cell detachment lowered local hydraulic conductance (Lp).

Maddox et al [2] have studied nephrotoxic serum nephritis at a later stage. In preliminary studies they found that by 33 to 35 days glomeruli were so severely damaged that micropuncture could not be performed. Thus, they chose to study the effects of a smaller dose of AGBM Ab 5 to 16 days after an injection of serum. In these rats, the glomerular filtration rate (GFR) was normal. Mean filtration pressure was elevated in nephritic rats as was plasma flow. Filtration pressure equilibrium was achieved in control but not nephritic rats. Ultrafiltration coefficient in nephritic rats was decreased when compared to control. It appeared that a marked reduction in the number of patent capillary loops played a role in lowering K_f in this model.

Data from studies in which indirect estimates of P_G were used to calculate K_f or LpA also suggested that the ultrafiltration coefficient was reduced in established AGBM or membranous nephritis [3, 16]. In these studies, decreased GFR occurred despite normal renal perfusion and normal or increased filtration pressure. Filtration equilibrium was not observed. It appeared that the filtration characteristics of the glomeruli were altered either by a decreased glomerular permeability or impaired glomerular capillary blood flow [3].

Our study proved to be unique in several respects. First, renal insufficiency was present although the histologic lesion was mild. Second, estimation of K_f was performed in vitro. Filtration was induced by applying an oncotic gradient across the capillary wall of an isolated glomerulus. Thus the effects of variations in glomerular perfusion and intratubular pressures were eliminated. Third, resistance to erythrocyte movement, an index of patency of glomerular capillary loops, was estimated by direct visual observation. The use of visual observation of glomerular filtration and erythrocyte ejection in vitro permits direct estimation of intrinsic glomerular physiologic and rheologic properties which has not been possible in in situ studies.

Results from our in vitro studies of glomerular function differ from the published results in demonstrating normal K_f in glomeruli from nephritic rats despite the presence of renal insufficiency. The discrepancy between the low K_f in previous studies and the normal K_f in our studies may occur either because of the nature of the lesion which we studied or because of the techniques we employed for estimating K_f. The glomeruli which we studied were affected by a lesion with few acute inflammatory changes and little complement localization. This histologic picture is in contrast to the acute inflammatory injury of nephrotoxic serum nephritis or the membranous lesion of Heyman nephritis [2, 4, 15, 16]. Blantz, Tucker, and Wilson [15] have shown that vasoactive substances released during acute inflammation produced arteriolar constriction in acute anti-GBM nephritis. It is possible that vasoactive substances may contribute to alterations in K_f through their actions on mesangial or other glomerular cells [17]. This postulate seems reasonable because humoral agents including angiotensin, PTH, and histamine also decrease K_f in in vivo studies [18–20]. We think that K_f may remain normal in the histologically less severe nephritis which we studied because the effects of vasoactive substances were minimal. Additionally, Blantz, Tucker, and Wilson have postulated that the hydraulic conductivity per area may be decreased in nephritis because of lengthening of the path for filtration by cellular infiltration or by loss of fenestrae. Loss of fenestrae of glomerular capillary endothelial cells has been documented by scanning electron microscopy after administration of nephrotoxins [21, 22]. K_f may remain normal in glomeruli which we studied because infiltration or loss of fenestrae were less marked than in acute AGBM nephritis.

Alternatively, the differences between our findings and those of previous studies may result from the different methods used to estimate K_f. In the usual in vivo method, K_f is influenced by the hydraulic conductivity per unit area of the capillary wall (Lp) and by the surface area available for filtration (A). The effective filtration area, in turn, is determined by the anatomic surface area of the capillary loops and the patency of the capillary lumens. Because glomeruli are composed of numerous channels which branch from the afferent arteriole and join near the efferent arteriole, partial obstruction of some channels may shunt blood through other channels. Patent capillary segments may be relatively overperfused and in filtration disequilibrium; a further increase in perfusion will not increase filtration. Partially obstructed capillary segments may remain poorly perfused and attain filtration equilibrium. In these segments filtration will be proportional to the low perfusion rate. Thus, partial or segmental capillary obstruction may lead to a decrease in effective filtration in vivo and to a decrease in K_f calculated from micropuncture estimates of glomerular pressures and single nephron filtration rates. Capillary obstruction has been cited by Maddox et al as a likely cause for decreased K_f in AGBM nephritis [2].

In contrast to the micropuncture approach, the in vitro method for estimating K_f uses the rate of expansion of capillary lumens as inward filtration occurs across the whole exposed surface of glomerulus. The exposed surface may be less than the entire anatomically available capillary surface area. Nevertheless, fluxes across the exposed capillaries may be used for comparing hydraulic conductivity of glomeruli from control and experimental kidneys. As in in vivo experiments, K_f is proportional to Lp and A, but, because transcapillary filtration is estimated from capillary expansion prior to the beginning of linear flow along the capillary, our estimates of K_f are relatively unaffected by partial occlusion of capillary segments as long as

the capillary lumens contain blood. The current in vitro method may permit us to estimate a K_f which is representative of the filtration capacity of the glomerulus regardless of effective perfusion, while in vivo estimates reflect the regional perfusion of the glomerulus as well as its intrinsic filtration properties.

Direct glomerular puncture cannot be performed in the Buffalo rats which are susceptible to the specific form of nephritis which we studied because they do not have surface glomeruli. Thus, we can only speculate about the contribution of hemodynamic factors to the progressive renal failure which we observed. Either decreased nephron perfusion, decreased glomerular capillary pressure, or increased intratubular pressure might result in decreased filtration. Data from previous studies of glomerulonephritis indicate that these are not present in other models of chronic glomerulonephritis [2, 13]. On the contrary, after an initial period of vasoconstriction, renal perfusion and glomerular pressures remain normal or elevated and tend to maintain glomerular filtration. The divergence between the decreased K_f observed by others and the normal values which we observed, coupled with our observations of impaired capillary erythrocyte flow, strongly suggest that segmental obstruction to capillary blood flow may be a major impediment to normal filtration in glomerulonephritis. Our findings suggest that Lp may be normal in this model while A may be reduced by underperfusion of certain capillary loops. Further studies in which both in vivo and in vitro measurements of glomerular filtration can be made are needed to establish the role of altered glomerular capillary perfusion in other models of glomerulonephritis.

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