PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 324–334 (1978)

Proteinuria and the Fragility of Normal and Diseased Glomerular Basement Membrane[1] (40342)

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There is a considerable body of knowledge with respect to the physical properties of interstitial collagen (1). It has been only recently, however that studies have appeared dealing with some of these properties in related basement membranes (BM). These are made up of collagen-like units bonded with sialoglycopeptides (2). The studies have been performed on two normal epithelial BM. Welling and Grantham dealt with the hydrostatic and osmotic conductance of isolated closed and perfused intact renal tubular segments and of such segments with the inner epithelial lining removed by the use of sodium desoxycholate. Tubular BM was found to be a relatively tough elastic structure (3). Likewise, Fisher and Wakely found the anterior lens capsule to be elastic and in fact at low stress values comparable to that of lightly vulcanized rubber (4). In both studies the authors found that the modulus of elasticity of BM was similar to that of interstitial collagen. Gelman and coworkers determined the melting temperature of collagen isolated from lens capsule by peptic digestion and found it to be significantly higher than that for interstitial collagen. They related this difference to the higher hydroxyproline content of BM collagen (5).

Direct measurements of the physical properties of vascular BM have not been reported. Access to vascular BM has to be at a capillary level. Only the capillaries of the renal glomerulus in their peripheral portions form free loops unencumbered by additional surrounding tissues. These loops are made up of a thick BM lined by a thin fenestrated endothelium on one surface and an epithelium with podocytic attachments on the opposite surface. The loops are held in place by inner attachment to a delicate mesangium. The closest approximation to an isolate of glomerular basement membrane (GBM) unaltered by the harsh methods necessary to obtain it in pure form, was to cut-off the outermost portions of the tufts with their free loops from freshly obtained glomeruli and then by micromanipulative techniques to detach portions of the loops from their mesangial connections. In so doing, we were able to convert a portion of a loop into a single straight strand 60–100 μm in length.

GBM has been compared to a thixotropic gel. It has been conjectured that in glomerular disease where there is increased permeability of the glomerular filter, this gel undergoes a physical change and becomes more sol-like. In fact Huang and coworkers (6) found that GBM from normal kidneys packed into a chromatographic column behaved like a highly cross-linked gel such as sephadex. GBM obtained from kidneys with nephrotoxic serum nephritis on the other hand appeared to be a more porous or less cross-linked gel. Somewhat similar results were obtained by Igarashi and coworkers (7).

It was of interest therefore to determine some of the physical properties of normal glomerular stands and to compare these values with those obtained from diseased glomeruli. Owing to technical limitations, the findings we are reporting deal with the effects of strain on the strands in terms of elasticity and fragility.

Procedures and methods. Male Lewis rats obtained from Microbiological Associates were used in all the experiments. However the GBM for the production of rabbit anti-rat GBM serum (NTS) was obtained from Holtzman rats. NTS was prepared in the manner described previously (8). In brief, male albino rabbits weighing 2.5 k were injected im with 100 mg GBM suspended in aluminum hydroxide gel. One-and-a half mil-

[1] Supported by U.S.P.H.S. Grant No. HE-01623.

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0037-9727/78/1593-0324$01.00/0
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liliters of the mixture were injected into each hind leg. The injections were repeated twice at weekly intervals. The rabbits were bled 21 days after the first injection. The serum was inactivated at 56° for 30 min and adsorbed three times for one hour each, with washed red blood cells from Holtzman rats. Normal rabbit serum (NRS) used for controls was inactivated and adsorbed in the same way.

Production of nephrotoxic serum nephritis (NTN). The rats weighed 150 g. They were injected iv with 2.0 ml NTS/100 g body wt. Control rats were injected with 2.0 ml NRS/100 g body wt. Urinary proteins were determined 6 and 18 hr after injection and on a daily basis thereafter. Renal biopsies were taken 6 and 24 hr and 10–15 days as well as 60 days after injection.

Production of aminonucleoside nephrosis (AMN). The rats weighed 90–120 g. They were injected SC daily for 7–10 days with 1.5 mg/100 g body wt of the aminonucleoside of puromycin as a 0.5% saline solution. Control rats were injected daily with an equivalent volume of saline. Urinary protein values were monitored daily. The food intake of the controls was adjusted to that of the nephrotic animals on a day-by-day basis. Renal biopsies were taken 8–11, 12–15, 25–28, and 33–39 days following the first injection.

Urinary proteins. The rats were placed in metabolic cages with access to rat chow and water. Urinary proteins were determined by a modified biuret assay (9). Baseline values were obtained prior to any experimental procedures. The values were expressed as mg protein/24 hrs/100 g body wt.

Preparation of microprobes. The microprobes were prepared by electrolysis of stainless steel wire, using a solution made up of equal parts of 3 *M* KCl and conc. HCl overlaid with a thin layer of xylene to prevent sputtering. The finely tipped probes were treated serially with 10% sodium bicarbonate, water, absolute ethanol and xylene. The probes were examined microscopically for an acceptable degree of fineness. By bending the wire before electrolysis, the tips could be made either curved or hooked (Fig. 1). Some of the tips of the probes were lightly dipped in unvulcanized rubber. Such rubber tipped probes could be used for holding or anchoring specimens.

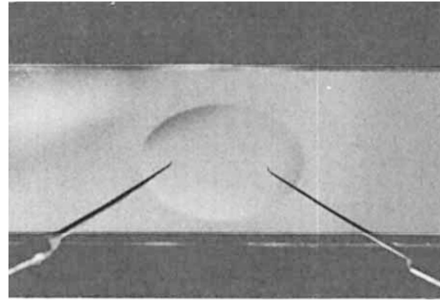

FIG. 1. Curved and hooked microprobes which were used to pluck capillary strands from the peripheral glomerular tufts. × 2.

Preparation of microdissection needles. The ends of No. 11 surgical blades were attached to applicator sticks with pyseal (Fisher Scientific).

Preparation of the renal specimens and determination of physical properties. The renal specimens were obtained by open biopsy. They were at once placed in ice-packed tubes containing 0.15 *M* NaCl. They were then lightly pressed between two glass slides and rinsed with saline into a small glass dish. Glomeruli were isolated from the suspension with dissection forceps and the use of a Bausch and Lomb stereoscope provided with 25X ocular, a 0.7 to 3.0X zoom lens and a 2X auxiliary lens. The isolated glomeruli were transferred to a slide-well containing fresh saline. With microdissection needles one was able to cut off the outermost portions of the tufts of the glomeruli with their peripheral loops. These tufts with their free loops were in turn transferred to the immersion well of a slide adjusted to the stage of a Bausch and Lomb microscope. The latter was equipped with 15X ocular and Leitz 20X and 32X objectives to provide an extra working distance between the objective lens and the immersion well on the stage of the microscope. Indirect lighting was used. The microprobes were attached to two Sensaur pneumatic de Fonbrune micromanipulators. The latter were arranged in relation to the microscope so that the microprobes could be immersed and manipulated within the saline of the well of the slide. A tuft was held taut at one end with one of the probes. One of the loops of the tuft was then grasped with a curved or hooked probe. By a quick pull on the appropriate micromanipulator a portion of the loop

could be detached from its mesangial attachment and drawn out as a single straight strand (Fig. 2). By retaining the hold on the strand, it could be stretched further either to a point where it still could recoil to its original length by releasing the strain upon it or it could be stretched to the point of rupture. The strand throughout these maneuvers remained single. In those instances where more than one loop had been grasped the extra ones slipped away from the main strand as it was being pulled out or stretched. Measurements of the initial length of the strand and the extent to which it could be stretched beyond the initial length were made with an ocular micrometer which had been calibrated with a stage micrometer. As control, pieces of rat tail tendon were cut on a freezing microtome to suitable lengths. These freshly shaved fragments were easily separated into single strands using the microprobes. The strands were treated in the same way as the glomerular ones.

Measurements were made on glomerular strands from the experimental animals with NTN and AMN and their appropriate controls and from normal untreated rats of various ages. In each instance four or five strands derived from different glomeruli of the biopsied specimen were tested. In addition we determined the effects of strain on normal glomerular strands exposed to a variety of agents which are listed in Tables III and IV. Some of these agents were selected to simulate the biologic ones thought to be effective in producing the altered changes in the physical qualities of the glomeruli derived from the experimental and aged animals.

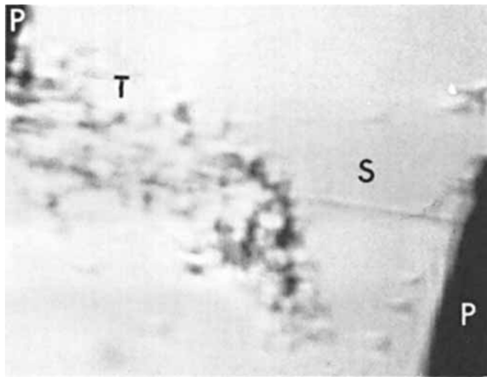

FIG. 2. A portion of a microprobe (P) is seen in the left upper corner. It anchors one edge of the peripheral glomerular tuft (T). Note the several capillary loops appearing as strands within the tuft. There are cells attached to them. The second microprobe (P) is seen at the lower right hand corner. It was used to pluck the single straight strand (S) which is stretched between it and the tuft. Note that the strand has practically no cells attached to it and seems to represent glomerular basement membrane only. × 560.

To verify that the alterations in the physical qualities of the glomerular strands from the experimental animals were not in vitro artifacts, the intact kidneys were perfused at normal and heightened pressures. The external surfaces of the perfused glomeruli were studied by scanning electronmicroscopy. The frequency of perforations as an index of increased fragility was sought for and compared with the perfused glomeruli from control kidneys.

Renal perfusion. Renal perfusion was performed on animals 24 hr after injection of NTS and on the 10th to the 12th day after the first injection of aminonucleoside. The animals were prepared by the iv injection of 200 units of heparin. Thirty minutes later they were perfused via the ascending thoracic aorta with 600 ml warm Ringer-Locke's solution pH 7.4, 288 mOsm/kg at 120 mm Hg. Subsequent perfusion was confined to the kidneys through the narrow sector of the abdominal aorta above and below the renal arteries. Branches of these arteries were tied as were the lumbar and spermatic arteries. The perfusion fluid was pooled human serum which had been kept chilled at all times. It was filtered through glass wool and centrifuged at 1800*g*. Cryoglobulins, if present were removed in a refrigerated ultracentrifuge at 30,000*g*. The serum prior to use was passed through a Seitz filter using a sterilizing pad with 0.4 μm pores. Both kidneys were perfused at 120 mm Hg for 7 min. The left renal pedicle was clamped and the right kidney perfused at 300 mm Hg for an additional 7 min. By judicious clamping of the renal pedicles the pressures could be maintained in these kidneys even when followed by reperfusion with Ringer-Locke's solution and subsequently with 2.5% glutaraldehyde in Sorenson's phosphate buffer pH 7.4, 395 mOsm/kg.

Light microscopy (LM) transmission (TEM) and scanning (SEM) electron microscopy. Tissues were fixed in Zenker's solution for LM. Sections were stained with hematoxylin and

eosin, periodic acid Schiff, Masson's trichrome and Lendrum's for fibrin. For TEM the material was fixed in 4% glutaraldehyde, post fixed in buffered 2% osmium tetroxide, dehydrated and embedded in Epon. Sorval "Porter-Blum" ultramicrotomes models MT-2 and MT-2B equipped with glass or diamond knives were used for sectioning. Sections were placed on parlodion and carbon coated 75 or 200 mesh copper grids or on uncoated 300 mesh copper grids. Grids were stained with uranyl acetate and lead citrate and examined with a Hitachi HS-7S or RCA EMU-4 electron microscope. Pieces of kidney perfused with glutaraldehyde were further fixed in this solution for 2 days and then dehydrated with acetone when being prepared for SEM. The specimens were then dried in a Bomar critical point dryer with CO_2. They were gold coated with a DC sputtering device to a thickness of approximately 25Å. The specimens were examined with a Cambridge stereoscan Mark 11A scanning electron microscope at an accelerating voltage of 20 KV.

Results. The terms used to describe the physical properties of the glomerular capillary strands are defined as follows. Stress is a resisting force set up in the strand by the externally applied force transmitted through the micromanipulators. However, this transmitted force was so small that it could not be detected by the most sensitive guages available to us. Strain is the change in shape that the strand underwent on applying stress. It was best expressed by the extent to which the strand could be stretched beyond its initial length. The percentage of the initial length beyond which the strand could be stretched and still retract to its original length was regarded as a measure of its elasticity. The percentage of the initial length beyond which the strand could be stretched to the point of rupture was regarded as a measure of its fragility. Hence a normal capillary strand could for example tolerate 60% strain before rupturing, that is a strand 60 μm in length could be stretched 36 μm for a total length of 96 μm. By contrast, a capillary strand from a diseased glomerulus ruptured as soon as it was stretched beyond its initial length and hence its tolerance was 0% strain. Between these two extremes one could use such terms as slight to marked increase in fragility. The elasticity and fragility of the strands could therefore be expressed in quantitative and qualitative terms without reference to the undetermined amount of stress. Valid comparisons could therefore be made between strands from normal and from diseased glomeruli.

The glomerular strands from untreated and treated control animals measured 60–100 μm in length. They were considerably elastic. They could be stretched up to 40% beyond their initial length with good recoil. However the strands would break if stretched from 60.2% to 77.1% beyond their initial length (Tables I–IV). By contrast single strands of rat tail tendon had little elasticity and broke when stretched to 12% beyond their initial length.

The elastic properties and the degree of fragility of the glomerular strands from the experimental animals were strikingly different. It was easier to isolate the glomerular strands from the animals with NTN and AMN than from the controls. There were

TABLE I. RELATION OF FRAGILITY OF GLOMERULAR STRANDS TO PROTEINURIA IN NEPHRITIC RATS.

Days after injection	With rabbit anti-rat GBM serum		With normal rabbit serum	
	Strain[a]	Urinary protein[b]	Strain[a]	Urinary protein[b]
0.25	4.4 ± 1.1 Median 0.0	70.7 ± 9.4	66.4 ± 2.1	6.2 ± 0.9
1.0	2.2 ± 2.7 Median 0.0	55.5 ± 11.7	60.2 ± 4.4	4.3 ± 2.3
10–15	5.2 ± 6.4 Median 0.0	40.0 ± 6.2	62.0 ± 4.2	4.4 ± 0.6
60	51.4 ± 10.2	1.5 ± 1.0	68.4 ± 4.8	4.1 ± 1.1

[a] The percentage of the initial length beyond which a glomerular strand could be stretched before breaking. The values are the mean ± SD of the measurements made on four or five strands from each of three rats. Medians are given for the rats treated with rabbit anti-rat GBM serum because of a skewed distribution of the values.

[b] Urinary protein is expressed as mg/24 hr/100 g body wt.

mucoid threads associated with the isolation of the glomerular strands most prominently from animals with NTN 6 hr after injection of NTS. Eighty-seven percent of the strands from the animals with NTN from 6 hr through the 10–15th day failed to tolerate any strain and broke immediately on stretching. Thirteen percent of the strands broke after tolerating a mean percentage strain of 32.4. The overall mean for all strands at any given time interval including those with 0% strain is given in Table I. In all instances, these changes in fragility coincided with pronounced proteinuria (Table I). In the case of the animals with AMN, all glomerular strands without exception broke immediately on stretching. Again this coincided with proteinuria as recorded 8–11, 12–15 and 25–28 days following the initial injection of the aminonucleoside (Table II). The glomerular strands both with NTN and AMN were so fragile that it was not possible to determine their elasticity. These changes were however reversible. With the diminution of proteinuria to normal values as observed 60 days after injection of NTS (Table I) and 33–39 days after administration of the aminonucleoside (Table II), the elasticity and fragility of the glomerular strands reverted to near normal values. However prior to the return to a normal urinary protein output random tests between the 15th and 60th day for NTN and the 28th and 33rd day for AMN indicated persistence of the high degree of fragility.

TABLE II. Relation of Fragility of Glomerular Strands to Proteinuria in Nephrotic Rats.

Days after the first injection	With aminonucleoside		With saline	
	Strain[a]	Urinary protein[b]	Strain[a]	Urinary protein[b]
8–11	0.0	20.2 ± 1.6	69.9 ± 1.2	3.3 ± 2.1
12–15	0.0	85.3 ± 4.1	66.0 ± 4.5	3.7 ± 1.1
25–28	0.0	18.5 ± 2.2	61.6 ± 0.8	2.8 ± 2.1
33–39	59.4 ± 3.2	5.3 ± 0.8	64.1 ± 5.3	3.5 ± 0.4

[a] The percentage of the initial length beyond which a glomerular strain could be stretched before breaking. The values are the mean ± SD of the measurements made on four or five strains for each of four rats.

[b] Urinary protein is expressed as mg/24 hr/100 g body wt.

The alteration in fragility of the glomerular strands was monitored by LM and EM studies of the glomeruli of the renal biopsies. At 6 hr with NTN there was some loosening of the mesangium and the deposition of electrolucent material beneath the endothelium. There was also dehiscence of the endothelium. Polymorphonuclear leukocytes and platelets were present in the capillary lumens. They were closely applied to the capillary walls. The foot-processes of the somewhat swollen visceral epithelial cells were still largely discrete. At 24 hr many of the glomerular capillary loops were thrombosed. In

TABLE III. The Fragility of Glomerular Strands Exposed to a Variety of Agents.

Agent[a]	Strain[b]
40% potassium iodide in buffered saline pH 7.4.	35.9 ± 1.2 (70.2 ± 2.5)
10% formalin in buffered saline pH 7.4.	5.0 ± 3.5 (69.7 ± 3.4)
0.1 mg/ml papain in EDTA pH 7.4.	7.6 ± 4.7 (72.1 ± 3.2)
0.1 mg/ml pronase in 1/15 *M* Sorenson's phosphate buffer pH 7.4.	0.0 (76.5 ± 4.1)
0.1 mg/ml collagenase in Tris buffer pH 7.5 or in 1/15 *M* phosphate buffer with 0.45% NaCl pH 7.4.	5.8 ± 3.6 (77.1 ± 3.7)
0.1 mg/ml Neuraminidase in Ringer-Locke with 1% bovine serum albumin pH 7.4.	25.9 ± 11.5 (66.7 ± 2.2)
0.1 mg/ml β-*N*-acetyl-D-glucosaminidase with 0.1 mg/ml bovine serum albumin and 0.01 *M* NaCl pH 7.4.	0.0 (75.0 ± 6.0)
0.1 mg/ml poly-l-lysine in Ringer-Locke pH 7.4.	0.0 (69.4 ± 3.2)
0.1 mg/ml protamine sulfate in Ringer-Locke pH 7.4.	1.3 ± 1.3 (69.4 ± 3.2)
0.1 mg/ml poly-l-glutamic acid in Ringer-Locke pH 7.4.	62.3 ± 8.7 (69.4 ± 3.2)
0.1 mg/ml heparin in Ringer-Locke pH 7.4.	66.4 ± 7.2 (69.4 ± 3.2)

[a] Twice crystallized papain, collagenase with <40 caseinase units/mg and neuraminidase with <0.1% proteolytic activity were obtained from Worthington Biochemical Corp. Pronase was obtained from Calbiochem. Poly-l-lysine, poly-l-glutamic acid and protamine sulfate were obtained from Sigma.

[b] The percentage of the initial length beyond which a glomerular strand could be stretched before breaking. The values are the mean ± SD of the measurements made on four or five strands. The figures in parenthesis are the mean ± SD of the values obtained by treating the glomerular strands with the buffer alone adjusted to the pH of the buffer plus agent.

TABLE IV. THE FRAGILITY OF GLOMERULAR STRANDS EXPOSED TO HISTAMINE AND 5-OH TRYPTAMINE.

Agent[a]	μg/ml	Strain[b]
Histamine base in Saline pH 7.0	1	51.8 ± 2.7 (64.6 ± 2.5)
Histamine base in Ringer Locke pH 7.4	1	48.2 ± 5.7 (66.7 ± 2.1)
Histamine base in Ringer Locke pH 7.4	3	42.0 ± 3.1 (66.7 ± 2.1)
Histamine base in Ringer Locke pH 7.4	5	37.3 ± 2.2 (66.7 ± 2.1)
Histamine base in Ringer Locke pH 7.8	7	34.6 ± 1.4 (67.3 ± 6.0)
Histamine base in Ringer Locke pH 8.0	50	39.4 ± 2.7 (64.7 ± 3.1)
Histamine base in Ringer Locke pH 8.0	100	23.2 ± 1.8 (64.7 ± 3.1)
Histamine base in Ringer Locke pH 8.1	150	0.0 (66.2 ± 3.3)
Histamine base in Ringer Locke pH 8.1	200	0.0 (66.2 ± 3.3)
Histamine Acid Phosphate in Tris buffer pH 7.5	7	48.3 ± 4.1 (67.2 ± 1.7)
Histamine Acid Phosphate in Tris buffer pH 7.4	50	32.3 ± 4.4 (71.0 ± 1.9)
Histamine Acid Phosphate in Tris buffer pH 7.3	140	18.8 ± 3.3 (68.8 ± 2.1)
5-OH tryptamine Creatinine Sulfate in Tris buffer pH 7.4	7	54.3 ± 4.3 (71.0 ± 1.9)
5-OH tryptamine Creatinine Sulfate in Tris buffer pH 7.3	50	44.6 ± 2.7 (68.8 ± 2.1)
5-OH tryptamine Creatinine Sulfate in Tris buffer pH 7.3	150	36.7 ± 2.9 (68.8 ± 2.1)
5-OH tryptamine Creatinine Sulfate in Tris buffer pH 7.3	200	27.2 ± 4.4 (68.8 ± 2.1)

[a] The histamine base was obtained from Pfanstiehl Chemical Co., Histamine acid phosphate from Eli Lilly Co. and 5-OH tryptamine Creatinine Sulfate from Sigma.
[b] The same as the footnote in Table III.

patent capillaries polymorphonuclears and platelets were still present and often apposed to bared BM. Foot processes were now irregularly approximated. The mesangial and BM changes were the same as at 6 hr but better defined. There might be some swelling of the lamina densa of the BM. Subsequent periods revealed subsidence of the inflammatory reaction, regeneration of the endothelium approximated foot processes and some GBM and mesangial thickening. There was a return to a more normal appearing glomerulus at 60 days at a time when urinary protein output and the physical properties of the capillary strands had returned to near normal. With AMN the changes during the period of proteinuria consisted of total approximation of foot processes. Occasionally there was dehiscence of these and their vacuolated visceral epithelial cells. There was no more than some thickening of GBM and an increase in mesangial matrix. At no time was there evidence for an inflammatory component comparable to that seen with NTN. With reversion to a normal urinary protein output, the glomeruli assumed a more normal appearance with largely discrete foot processes and the capillary strands derived from them resumed near normal physical properties.

The glomerular strands of untreated rats were examined periodically as they aged. These invariably gave normal values for fragility and elasticity. With beginning proteinuria at 2.5 years of age the values of strain in two survivors decreased from normal values to a mean of 40.6 and 43.8%. At this time 12–15% of the glomeruli by LM had segmental or rarely total sclerotic changes. In the ensuing 4 weeks the values of strain dropped to a mean of 15.3%. There was a distinct decrease in elasticity. The glomerular strands failed to return to their original length or did not recoil at all, when stretched below their breaking point. Thirty percent of the glomeruli now showed marked sclerosis. These latter when isolated had yellow patches presumably representing the sclerotic loops. Strands from these loops were highly fragile and broke immediately. Less involved glomeruli had GBM and mesangial thickening and by EM fusion of foot processes over thickened loops.

The increased fragility of the diseased glomerular capillaries could be corroborated by perfusion of the kidneys and the examination of their glomeruli by SEM. Ninety-three glomeruli from 9 control kidneys perfused at 120 or 300 mm Hg showed no perforations except in one kidney perfused at 300 mm where three out of ten presented single perforations. In the case of NTN of 43 glomeruli from four kidneys perfused at 120 mm 13 or 30% showed one or more perforations. Of 63 glo-

meruli from five kidneys perfused at 300 mm 19 or 30% showed one or more perforations. With AMN of 33 glomeruli from three kidneys perfused at 120 mm 19 or 57% showed one or more perforations. At 300 mm Hg of 45 glomeruli from four kidneys 32 or 71% showed perforations (Figs. 3–5).

The effects of a variety of agents on glomerular strands isolated from normal glomeruli are presented in Tables III and IV. It is to be emphasized that these results represent changes in fragility which occurred at room temperature and often within minutes following exposure to the agent. The buffers used as controls were adjusted to the same pH as the buffer plus the agent. Forty percent KI which causes chemical contraction of collagen comparable to heat contraction increased the fragility of the glomerular strands. There is even a greater increase in fragility following fixation of the strands in 10% formalin. The proteolytic enzymes, papain, pronase and collagenase all sharply and markedly increased fragility. Neuraminidase and β-*N*-acetyl-D-glucosaminidase both increased fragility. Neuraminic acid and N-acetyl glucosamine are components of the noncollagenous glycopeptides of GBM. However the striking effects of the glucosaminidase might have been due to a possible minor contaminant with a proteolytic enzyme. It is of extreme interest that by contrast with the polyanions, poly-l-glutamic acid and heparin which had no effect on the glomerular strands, the polycations, poly-l-lysine and protamine sulfate produced marked increases in fragility. Histamine and 5-OH-tryptamine both highly vasoactive substances, likewise increased the fragility of the glomerular strands. The increase was a graded one commensurate with the increasing concentration of the agent.

Discussion. Of the components of the wall of the glomerular capillary it is the BM which forms the structural backbone and serves as its support. In fact Murphy and Johnson (10) have submitted the data of Welling and Grantham (3) on renal tubular BM to mathematical analysis and have drawn the inference that capillary BM and particularly GBM are responsible for the rigidity and self support of these vessels. The results obtained

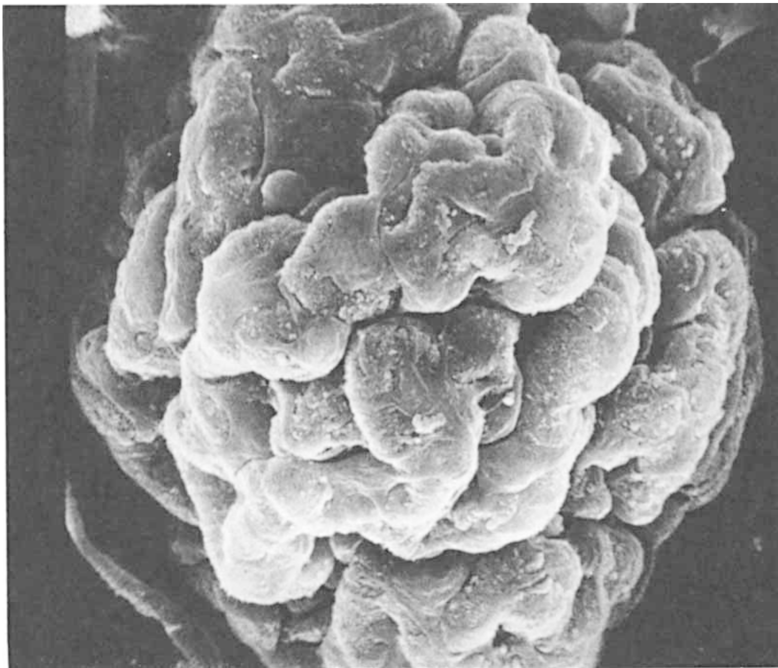

FIG. 3. Scanning electronmicroscopic view of a glomerulus from a normal kidney perfused with serum at 300 mm Hg. Details of the visceral epithelial cells and their processes can readily be made out. There are scattered microvilli. There are no perforations. × 1000.

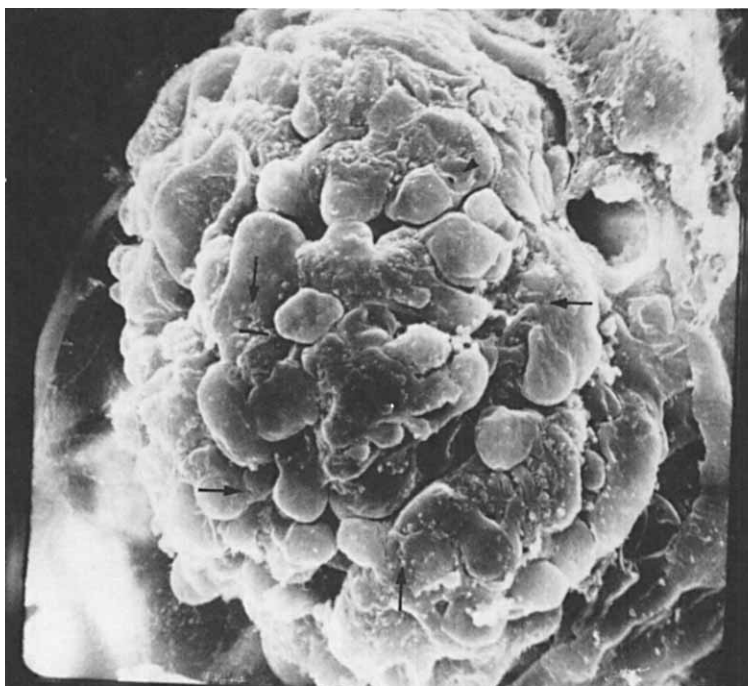

FIG. 4. Scanning electronmicroscopic view of a glomerulus from a kidney with aminonucleoside nephrosis perfused with serum at 300 mm Hg. The loops are irregular and distorted. Cells and their processes are largely obliterated. The surfaces tend to be smooth with some microvilli and some blebs. There are multiple perforations indicated by the arrow head and arrows. × 1000.

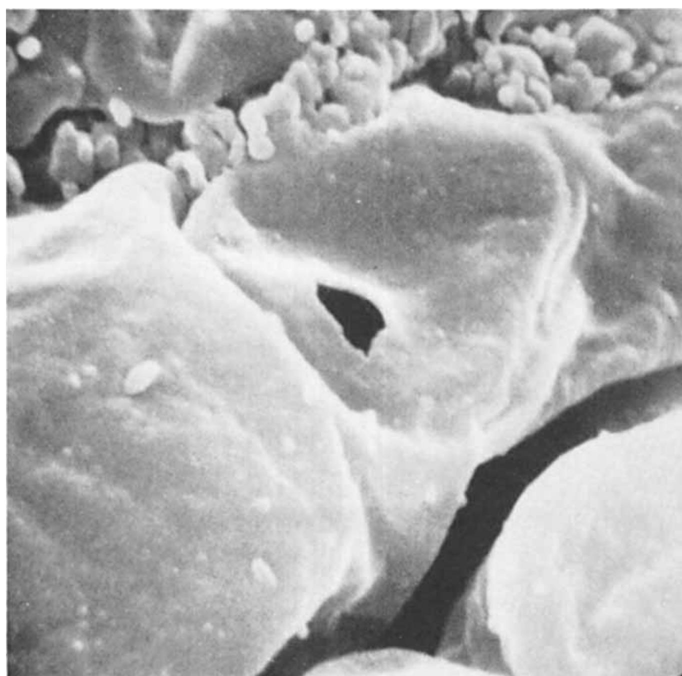

FIG. 5. As in Fig. 4. The perforation indicated by the arrow head in Fig. 4 is shown in an enlargement of the area. × 10,000.

with the isolated single strands from peripheral glomerular capillaries can therefore be considered as representative of the physical properties of GBM.

Normal glomerular strands are very elastic and are resistant to rupture when stressed not unlike that reported for renal tubular BM (3) and anterior lens capsule (4). By contrast there is a marked increase in the fragility of the strands from the glomeruli of rats with NTN and AMN and from the affected glomeruli in spontaneous glomerulosclerosis. The degree of increase in fragility was such that elasticity could no longer be measured since the strands broke immediately on being stretched beyond their initial lengths. The strands from very aged rats were less elastic then normal. The changes in fragility occurred in all instances simultaneously with the onset and persistence of proteinuria. They reverted however to near normal during the recovery phase of NTN and AMN with the resumption of a normal output of urinary protein. Comparable degrees of increased fragility were observed *in vitro* by brief exposure of normal glomerular strands to proteolytic and collagenolytic enzymes, to neuraminidase and to polycations. Polyanions by contrast were without effect as were the various buffers that were used as vehicles for all these agents.

The degree of increase in fragility of the glomerular strands in NTN appeared to be somewhat variable. Not all strands broke immediately on stretching, suggesting that the damage to the capillary wall was not uniform and that the immune inflammatory response was more intense in focal glomeruli and in segmental sectors. This seemed to be borne out by the perfusion studies where a fixed 30% of the glomeruli showed perforations independent of the pressure employed whether at 120 or 300 mm Hg. The damage to GBM is assumed to occur through the release of lysosomal enzymes particularly from the polymorphonuclear neutrophils of the inflammatory exudate (11). As indicated in the *in vitro* experiments even brief exposure of the glomerular capillary strands to somewhat similar enzymes could bring about a sharp increase in their fragility. In addition to enzymatic action there is a change in the staining pattern for the anionically charged sialoglycoproteins of the glomerular capillary wall shortly after the onset of NTN followed by a quantitative decrease in sialic acid (12). Considerable emphasis has been placed recently on the reduction of the normal anionic charge of the glomerular capillary wall with reference to increased permeability of anionically charged serum proteins such as albumin (13). In fact perfusion of the kidney with polycations can lead to proteinuria (14) and as shown here exposure of normal glomerular strands to polycations can increase their fragility promptly and markedly. In effect therefore both reduction in net negative charge and enzymatic action appear to account for the increased permeability and fragility of the glomerular capillary wall in NTN.

The increased fragility of the glomerular strands with AMN was more uniform. All strands broke immediately on being stretched beyond their initial length. Also the number of glomeruli with ruptures following perfusion was greater than with NTN and increased from 57% at 120 mm Hg to 71% at 300 mm. There is no significant inflammatory component with AMN. However, there is loss of net negative charge associated with decrease in sialic acid (15). There is also a change in the composition of the GBM with a decrease in hydroxylysine and hydroxyproline, a corresponding increase in lysine and proline and an altered glucose–galactose–hydroxylysine ratio of 2:1:1 as compared with 1:1:1 for normal GBM (16). Altered synthesis of GBM as well as reduction in negative charge may be the basis for increased permeability and fragility in AMN.

Morphologic changes in GBM with NTN and AMN have been said to vary from none to some edematous swelling in the earlier stages and to some thickening in the later stages. This is borne out by our own observations. It is not clear to what extent such changes in and of themselves contributed to increased permeability and fragility. However with spontaneous glomerulosclerosis there is variable and in the most affected loops marked GBM thickening. There are no data to indicate whether such thickened BM are associated with loss of net negative charge or with distinctive changes in chemical composition. It is known that with aging there is increased hydroxylation of lysine of the GBM

and increased glycosylation of hydroxylysine (17). There is also a decrease in sialic acid (18). Nonetheless morphologically altered GBM in glomerulosclerosis is associated with increased fragility and presumably with increased permeability.

It would appear therefore that besides direct enzymatic action, alterations in the chemical composition and molecular configuration of GBM including a reduction in its net negative charge can bring about striking changes in its fragility. These changes appear to be intimately associated with increased permeability to plasma proteins. With proteinuria there is commonly approximation or "fusion" of foot processes with displacement of the slit diaphragms. This is almost universal with AMN, more irregular with NTN and occurs over thickened loops in glomerulosclerosis. It has been suggested by Seiler and coworkers (19, 20) that the mobility of the foot processes may be primarily dependent upon altered charge relationships between individual foot processes and between foot process and BM. With the movement of the foot processes the slit diaphragms would then be displaced. One wonders however to what extent an increasingly fragile GBM whether induced by altered charge or not would lead to loosening and displacement not so much of the foot processes as of the film-like slit diaphragms. One would then be dealing, in essence with the same attempt on the part of the visceral epithelial cell to cover the denuded GBM as in the case of the elongation and extension of a regenerating epithelial cell over an ulcerated surface. The movement of the foot processes is associated with the displacement of the glycocalyceal coat from the interpedicular surfaces and the slit diaphragms. It is entirely possible that the strings of mucoid material encountered in the preparations of glomerular strands particularly the ones 6 hr after the injection of NTS may be derived from such displaced glycocalyces perhaps rendered more mucoid by the action of enzymes which have permeated through the GBM or by reduction in its negative charge.

Changes in the fragility of the glomerular strands can be brought about not only with cationic polyamines but with simpler basic amines such as histamine and 5-OH-tryptamine. This is of interest since it is possible that these two vasoactive substances can be released from mast cells and/or platelets in sufficient concentration so as to bring about comparable changes in fragility of capillary and venular BM. The increased permeability observed with these amines would be due therefore not only to disjunction of the endothelial cells allowing the vascular contents to come in contact with the BM but would also be due to the altered physical property and presumably permeability of the BM itself. The vasoactive cationic polypeptides released from the lysosomes of polymorphonuclears and the basic kinins may act on the BM in the same way.

Summary. Single straight capillary strands measuring 60–100 μm were secured by micromanipulators and microprobes from excised peripheral portions of the tufts of isolated glomeruli. The physical properties of these strands were considered to represent those of GBM. Normal glomerular strands could be stretched up to 40% beyond their initial length with good recoil but broke when they were stretched from 60 to 77% beyond their initial length. By contrast 100% of the glomerular strands from the kidneys with AMN, 87% of those from kidneys with NTN and the most affected glomeruli from aged rats with glomerulosclerosis broke immediately when stretched beyond their initial length. Elasticity could not be determined under these circumstances. Normal glomerular strands showed marked increases in fragility when briefly exposed to proteolytic enzymes, neuraminidase, to polycations and to basic amines. It seems that direct enzymatic action on GBM or alterations in its chemical composition and molecular configuration as well as a reduction in its net negative charge can bring about striking changes in its fragility. These changes appear to be intimately associated with increased permeability. They appear with the onset of proteinuria in AMN and NTN and they return to near normal when the output of urinary protein returns to normal. It is suggested that the approximation of foot processes which commonly accompanies proteinuric states is a response on the part of the visceral epithelial cell to the instability and displacement of the foot processes and in particular of the delicate slit

diaphragms occasioned by an underlying increasingly fragile BM. It is also suggested that the vasoactive amines not only lead to disjunction of endothelial cells but render the capillary or venular BM increasingly fragile and permeable.

The authors are greatly indebted to Dr. B. Weissman for the supply of β-*N*-acetyl-D-glucosaminidase and to Dr. E. H. Polley for his advice on how to prepare the microprobes.

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Received March 10, 1978. P.S.E.B.M. 1978, Vol. 159.