Alterations in Glomerular Permeability In Streptozotocin-Induced Diabetic Rats

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The alteration in glomerular basement membrane permeability associated with microangiopathy in streptozotocin-induced diabetic rats was studied by determining the movement across the glomerular basement membrane of anionic ferritin probes injected into rats at different points in the development of the disease. Visualization of the concentration gradient of anionic ferritin and changes in ultrastructure was accomplished by electron microscopic examination of renal tissue prepared from both control and diabetic rats. In all control rats, the anionic ferritin did not leave the glomerular capillary lumen, nor were there any changes in the normal morphology of the glomerular capillary wall. In the diabetic animals, the concentration of anionic ferritin shifted from the capillary lumen in the abluminal direction. Distinct morphologic changes, such as widening of endothelial intercellular junctions, focal detachment of podocyte foot processes, and extensive thickening of the glomerular basement membrane, were noted in the diabetic rat, and these changes appear to correlate with the observed increase in permselectivity of anionic ferritin across the glomerular capillary wall.

The authors have described the microscopic anatomy of the glomerular basement membrane in both the healthy individual and in the patient with diabetes.[1] It was further explained how pathologic changes in the glomerular basement membrane because of alterations in its chemical composition caused by diabetes mellitus adversely affected its role in permselectivity when dealing with plasma proteins. These changes are now described experimentally using the streptozotocin-induced diabetic rat as the animal model.

The endothelium of the microvasculature is composed of highly differentiated cells that help regulate the movement of macromolecules between vascular and extravascular spaces. Various components of the capillary wall have been associated with the permeability of macromolecules. These include plasmalemmal vesicles, transendothelial channels, intercellular junctions, and basement membranes.[2-5]

Historically, structural studies of capillary permeability have been performed by time-course studies of transendothelial passage of intravascularly injected probes of graded sizes and varied chemical nature.[6-10] More recent studies have shown that the restricted transport of polyanions smaller than albumin through the capillary wall is primarily caused by the electrical charge of the polyanions (small pore theory), while the movement of macromolecules larger than albumin from the capillary lumen through the subendothelial layers of the basement membrane is related both to electrical charge and molecular size (large pore theory).[11-15]

It has been well established that a significant increase in the microvascular permeability of albumin occurs at the level of the glomerular capillary

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wall in the patient with diabetes. This is found in both the human with diabetes and the experimentally induced animal model.[16-19] These studies, however, only addressed diabetic microangiopathy at the regional or whole organ level.

In order to understand this phenomenon better, it is necessary to investigate changes in permeability at the endothelial cell level with these questions in mind: 1) where do the changes occur, intracellularly, intercellularly or both; 2) when in the course of progression of diabetes do the changes appear; and 3) how rapidly do they occur once they develop?

Recent studies have shown the efficacy of using intravascularly injected probes of graded sizes to study the onset of changes in permeability over time.[20, 21] It was found that probes ranging in molecular diameter from 3.3 to 40 nm were differentially transported through the endothelium of myocardial capillaries.

The authors' study was designed to determine if the rate of permeability of a specific-sized macromolecule (anionic ferritin) through the glomerular capillary wall was altered during the progression from short-term to long-term diabetes in the streptozotocin-induced diabetic rat.

Materials and Methods

Animals

Female Sprague Dawley rats weighing 150 g to 175 g were used. All animals were maintained on a standard rat chow diet and water *ad libitum*. Diabetes was induced by the injection of 0.5 ml streptozotocin (65 mg per kg body weight dissolved in sterile saline) into the tail vein of ether-anesthetized animals. Control animals received a similar injection of sterile saline.

Experimental Protocol

Animals that had diabetes for 1 week, 15 weeks, or 52 weeks were used in this study. Anionic ferritin, pI 4.5, 2X, and cadmium free, was used as received from the supplier. Ether-anesthetized rats were administered anionic ferritin intravenously into a tail vein. Tracer dosage was 10 mg per 100 g body weight. This dosage is comparable with those used by investigators in similar mammalian studies.[22-24] Ether-anesthetized animals were sacrificed by decapitation either 1 min or 3 min after ferritin injection.

Electron Microscopy

Representative kidney tissue samples were immediately immersed in 3% paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer. All tissues were postfixed in 1% OsO_4 in 0.1 M phosphate buffer, *en bloc* stained with uranyl acetate in sodium maleate buffer, dehydrated in graded alcohols, and embedded in epoxy resin. Thick sections (1 to 2 µm) were stained with 1% toluidine blue in 0.1% sodium borate. Thin sections with silver interference colors were mounted on copper grids, stained with uranyl acetate, and lead citrate and then examined at 80 kV in an electron microscope.[25, 26]

Results

Control Animals

In all control animals, regardless of age or circulation time, anionic ferritin did not leave the glomerular capillary lumen, nor were there any changes in the normal morphology of the glomerular capillary wall.

Diabetic Animals

The 1-week, 1-min diabetic animals exhibited some movement of anionic ferritin from the capillary lumen into the glomerular basement membrane, while in the 1-week, 3-min animals, the concentration of anionic ferritin in the glomerular basement membrane increased, with a concomitant increase in its movement toward the abluminal surface of the glomerular basement membrane (Fig. 1). The only apparent change in the ultrastructure was a slight thickening of the glomerular basement membrane. In the 15-week, 1-min animals, anionic ferritin was distributed on a continuous gradient toward the luminal surface (Fig. 2). In both the 15-week, 3-min animals and the 52-week animals, the concentration of anionic ferritin had shifted in the abluminal direction (Fig. 3).

Distinct morphologic changes were noted in the 15-week and 52-week diabetic animals. These included marked widening of endothelial intercellular junctions, focal detachment of podocyte foot processes, and extensive thickening of the glomerular basement membrane (Fig. 3). These changes appear to correlate with the observed increase in permselectivity of anionic ferritin across the glomerular basement membrane.

Discussion

Glomerular basement membranes have been shown to be the primary filtration barrier in the mammalian kidney.[12] Kanwar and Farquhar[15] used cationized ferritin to establish that anionic binding sites are an intrinsic part of the mammalian glomerular basement

membrane and that these sites are composed mainly of heparan sulfate proteoglycan, a sulfated glycosaminoglycan possessing a high density of fixed negative charges which is responsible for the generation of an electrostatic permeability barrier.[27, 28] Proteinuria is one of the typical clinical signs of diabetes mellitus. It has been confirmed that this nephropathy is caused by a disruption in the charge barrier of the glomerular basement membrane.[29, 30] Several studies have shown that the heparan sulfate proteoglycan content of the glomerular basement membrane is altered in diabetes mellitus and that this is responsible for the disruptive perturbations in the permselective barrier.[31-34]

The authors' finding that a slight, but definite, increase in movement of anionic ferritin across the glomerular basement membrane in the 1-week diabetic animal, regardless of the circulation time, supports the observation by Moriya et al[35] that the charge barrier of the glomerular basement membrane is adversely affected within 1 week of streptozotocin induction. The results also confirm that movement of anionic ferritin through the glomerular basement membrane is controlled primarily by the electrostatic properties of the glomerular basement membrane and that this property is adversely affected in diabetes.

By 15 weeks, there was significant movement of anionic ferritin across the glomerular basement membrane, especially in the 3-min animals. This concurs with Moriya et al[35] who found that by 8 weeks after streptozotocin induction, the charge barrier of the glomerular basement membrane had been significantly disturbed with a concomitant appearance of albuminuria.

Another factor to consider in the development of proteinuria in diabetes is the changes in the morphologic and biochemical makeup of the glomerular basement membrane and the effect of these changes on the permeability characteristics of the glomerular basement membrane because of the size of the permanent macromolecules. Ota et al[36] observed that the glomerular basement membrane in nephrotic syndrome developed cavities that coalesced to form tunnels with diameters greater than the diameter of serum albumin molecules that would lead to proteinuria.

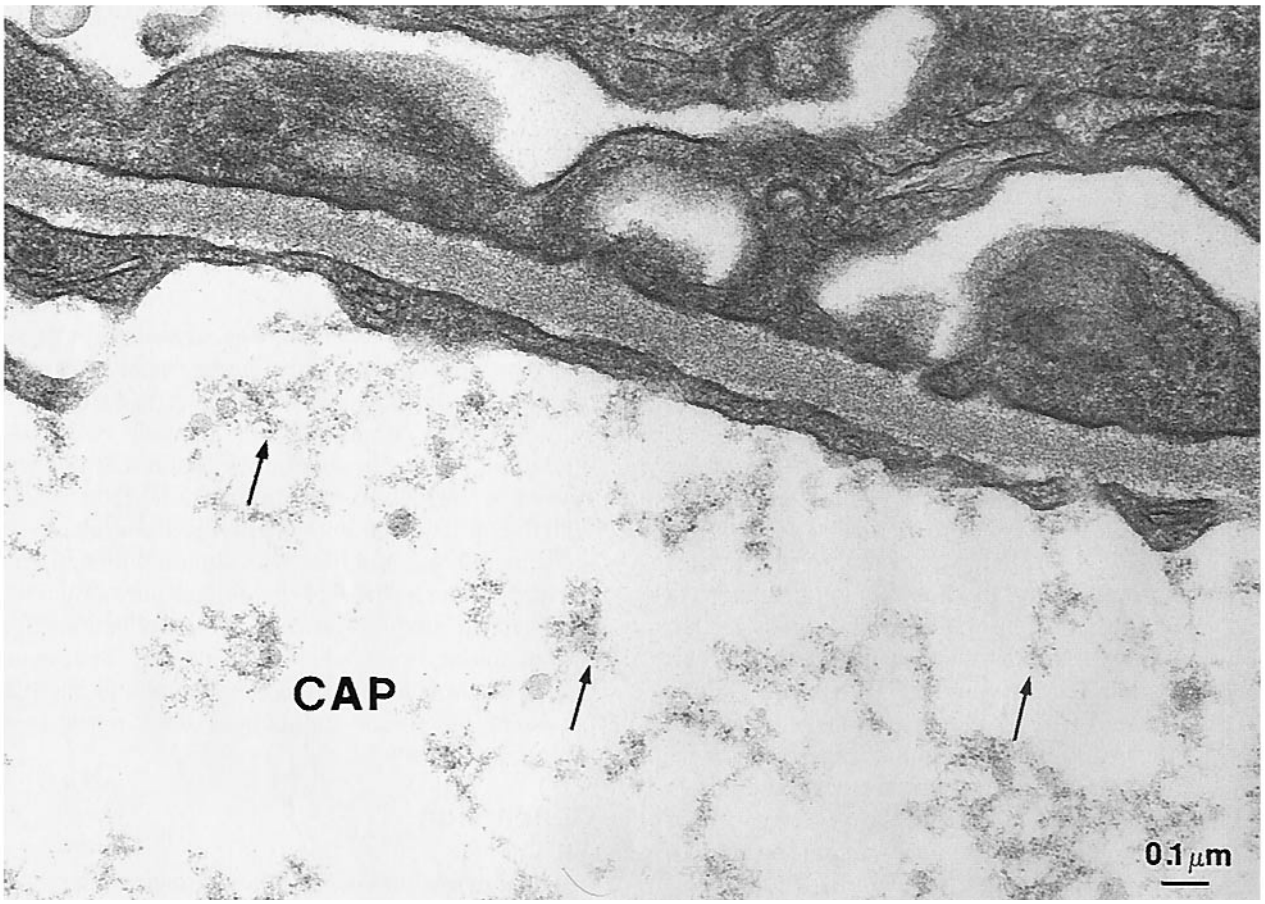

Figure 1. Electron micrograph of glomerular capillary of the streptozotocin-induced diabetic rat after 1 week. The animal was sacrificed at 1 min after infusion of anionic ferritin. Anionic ferritin (arrows) is located primarily in the capillary lumen (CAP). Note there are no significant alterations in glomerular capillary wall morphology (×59,000).

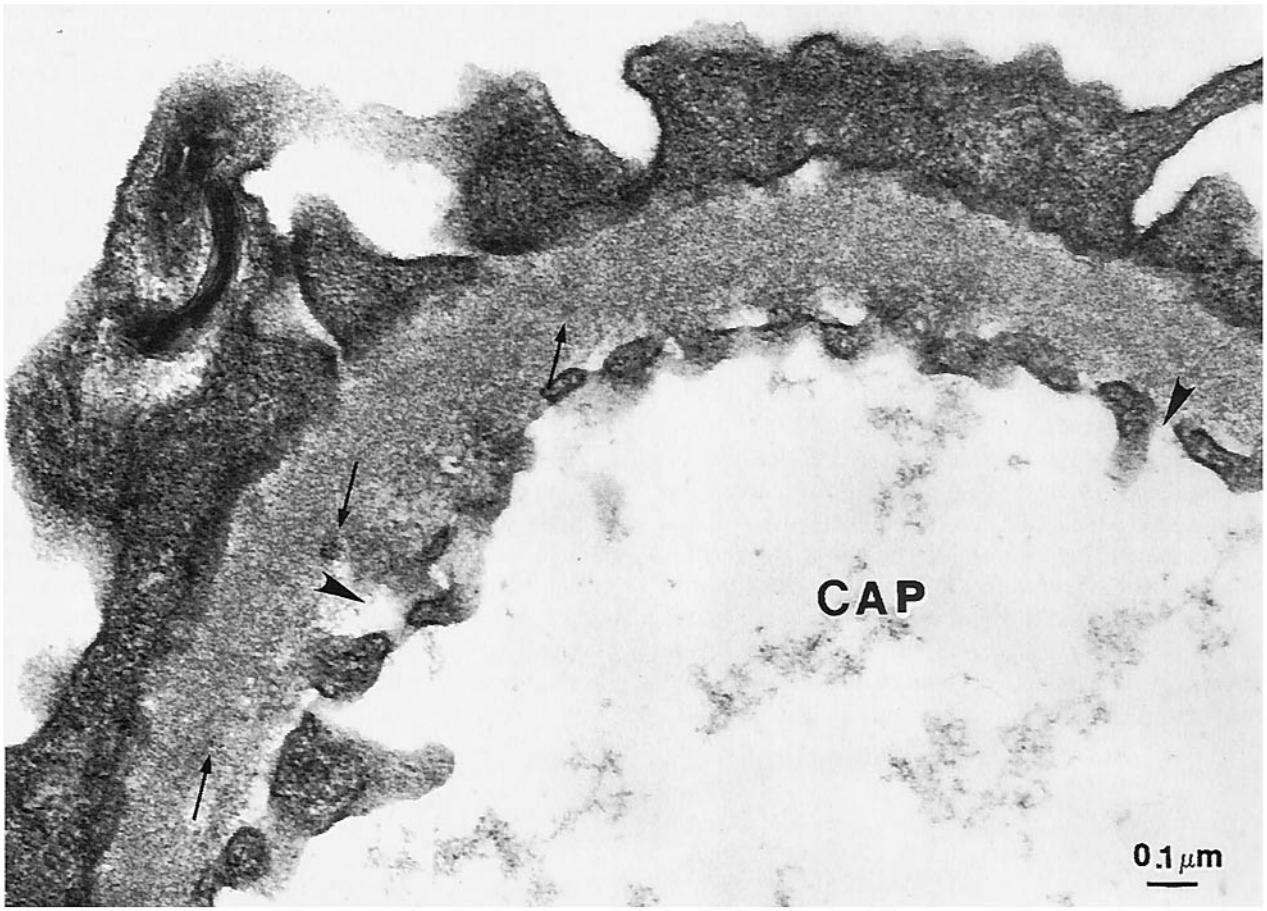

Figure 2. Electron micrograph of the glomerular capillary of a streptozotocin-induced diabetic rat after 15 weeks. The animal was sacrificed 1 min after infusion of anionic ferritin. Anionic ferritin (arrows) has moved into the glomerular capillary wall from the capillary lumen (CAP). The glomerular capillary wall shows morphologic changes (arrowheads) consistent with diabetic microvascular disease (× 59,000).

In diabetic nephropathy, quantitative alterations in type IV collagen, heparan sulfate, and laminin affect the structural integrity of the glomerular basement membrane and its biochemical integrity.[37-40] It is also known that all of these perturbations affect the porosity of the glomerular basement membrane and lead to an increase in proteinuria.[41] Ota et al[42] have found that the cavitation of the glomerular basement membrane in diabetic nephropathy is sufficient to disrupt the size barrier (large pore theory); they postulate that this disruption is sufficient to permit massive proteinuria.[42] Moreover, Gall et al[43] have found that an increase in large pore area of the glomerular basement membrane leads to increased proteinuria in the person with diabetes.

The observations of morphologic changes in the diabetic glomerular basement membrane by 15 weeks support the work of Østerby et al[44] who showed a correlation between glomerular lesions and advanced diabetic nephropathy. The possibility that the glomerular basement membrane pathology that the authors observed was experimentally induced as a result of the polyanionic tracer used must be considered. Kubosawa et al[45] described similar results after the administration of highly cationized ferritin in the rat. However, they found that this was caused by the electrostatic interaction of the cations with the anionic binding sites in the glomerular basement membrane and also with the anionic cell coat present on the surface of the glomerular podocytes. In any of the studies known to have used anionic ferritin or anionic horseradish peroxidase in either mammalian or nonmammalian kidneys, similar glomerular basement membrane pathologies have never been reported.[11, 13, 14, 28, 46, 47]

Conclusion

Vascular permeability of the glomerular capillary wall in the streptozotocin-induced diabetic rat increased significantly as the disease developed from 1 week to 52 weeks' duration. This change in permeability was

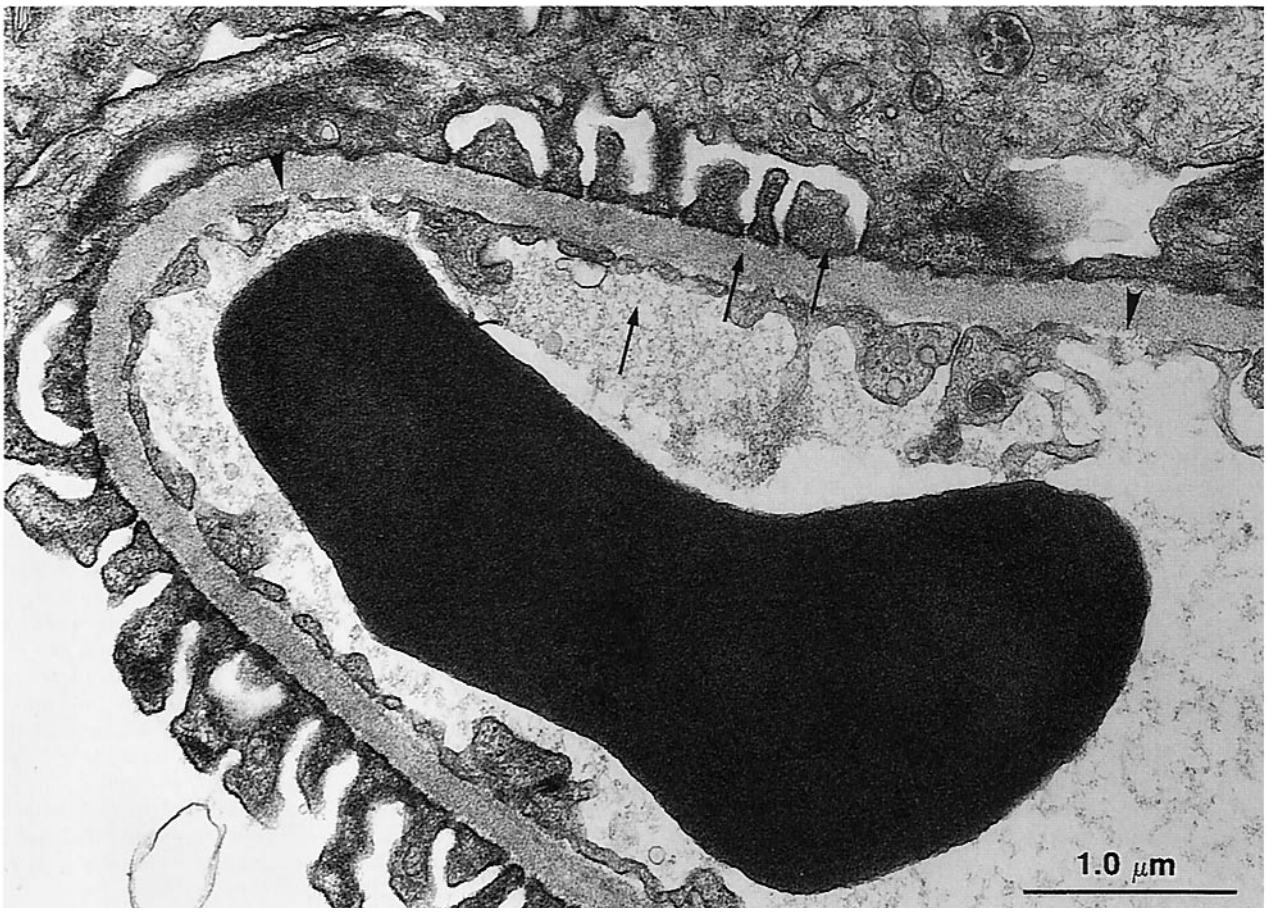

Figure 3. Electron micrograph of the glomerular capillary of a streptozotocin-induced diabetic rat after 1 year. The animal was sacrificed 1 min after infusion of anionic ferritin. Anionic ferritin (arrows) is distributed across the glomerular capillary wall, which shows morphologic changes (arrowheads) more pronounced than was seen in the 15-week diabetic animal (× 26,900).

indicated by the rapid movement of anionic ferritin from the glomerular capillary lumen through the glomerular basement membrane and into the urinary space of Bowman's capsule. Distinct morphologic changes in the glomerular capillary wall were evident in the 15-week and 52-week animals. These included thickening of the glomerular basement membrane, focal detachment of podocyte foot processes, and widening of endothelial cell junctions.

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