

BASAL LAMINA HETEROGENEITY IN THE GLOMERULAR CAPILLARY TUFTS OF HUMAN KIDNEYS*

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The basal laminae are a family of ubiquitous extracellular materials investing individual cells or enveloping nests of epithelial cells (1, 2). They are synthesized by the cells which they invest (3-6). Chemical (7, 8), as well as immunochemical (9-11) data indicate heterogeneity of basal laminae synthesized by different types of cells. The chemical and structural heterogeneity may be responsible for the diverse physiologic roles played by various basal laminae in different organ systems.

In glomerular capillary tufts of kidneys, there are three distinct cell types (epithelium, endothelium, and mesangial cells) closely associated with a continuous basal lamina scaffold. The glomerular capillary basal lamina is shared on one side by the endothelium and the other by the epithelium. The relative contribution of each of these three cell types to the synthesis of glomerular basal lamina is unknown. Except for the basal lamina immediately surrounding mesangial cells (mesangial matrix), which appears less compact in electron microscopy (12), there is no direct evidence that the glomerular basal lamina is heterogeneous. The question of basal lamina heterogeneity may be important in our understanding of glomerular physiology, as well as the glomerular reaction to injury.

A novel approach to the analysis of composite epithelial and endothelial basal laminae has been developed in our laboratory (13). The method involves immersion of fresh human tissues in a 5-M guanidine solution before fixation for electron microscopy. The guanidine treatment causes differential swelling and a decrease in the electron opacity of the endothelial basal lamina. The results make the latter readily distinguishable from the epithelial basal lamina. Applied to human kidneys, the guanidine treatment technique also clearly delineates a less electron-opaque basal lamina associated with the endothelium and the mesangium from that associated with the epithelium. The results provide a new insight into structural organization and function of glomerular basal laminae.

Materials and Methods

Three surgically removed kidneys obtained from Pathology Service of the Veterans Administration Medical Center, Seattle, Wash., were used in the studies. Two kidneys were removed for hemorrhagic cysts from patients 56 and 62 yr of age. The third kidney was from a 68-yr-old patient with renal cell carcinoma. None of the patients showed clinical evidence of glomerular diseases. Immediately after surgical removal, samples of the renal cortex from normal-appearing portions of kidneys were diced into 1-2-mm cubes. One part was fixed in 3% glutaraldehyde in

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0.1 M cacodylate buffer, pH 7.4. The rest were immersed in 5 M guanidine HCl, pH adjusted to 7.0–7.2 with 0.1 N NaOH, at 20°C for 24 h. After a brief rinse in phosphate-buffered saline, the specimens were fixed in the same glutaraldehyde fixative for 4 h. The glutaraldehyde-fixed tissues were processed for electron microscopy as previously described (13).

For histologic preparations, renal tissues were fixed in 10% buffered (pH 7.4) formalin solution. Paraffin sections were cut at 2 μ m thicknesses and stained with hematoxylin-eosin, periodic acid Schiff reagent, and periodic acid methanamine silver reagent (14).

Results

Light microscopy of histologic sections stained with various stains revealed normal renal structures, except for minimal benign nephrosclerosis as expected of patients at this age group. Electron microscopy of tissues fixed with glutaraldehyde without guanidine treatment also disclosed normal glomerular structures.

Treatment with a 5-M guanidine solution caused complete effacement of the cellular structure. Nuclear contents became globules of fibrillar material. Membranes were fragmented into vesicles of varying sizes and dispersed in a background of finely granular materials. The latter presumably represented cellular proteins, solubilized and denatured by guanidine. However, the glomerular basal lamina, which was composed of an electron-opaque epithelial basal lamina and a relatively electron-lucent endothelial-mesangial basal lamina, remained intact.

The epithelial basal lamina was the most prominent element of the glomerular basal lamina scaffold. It appeared as a continuous sheetlike structure within each glomerulus and merged imperceptibly with the parietal epithelial basal lamina of Bowman's capsule at the vascular pole. It first folded into several vascular tufts in a glomerulus (Fig. 1). Within each tuft, the epithelial basal lamina further folded into loops of capillaries but never completely encircled the entire circumference of each capillary (Figs. 1 and 2). Partition of the vascular space defined by the epithelial basal lamina into individual capillary lumen was accomplished by mesangial basal lamina (Fig. 2). Thus, two groups of glomerular capillaries were identified. Peripheral capillaries were those with only one mesangial region along the entire circumference. Capillaries contained two or more mesangial areas along their circumferences were designated axial capillaries, because they were generally seen in the axial portions of capillary tufts (Figs. 1 and 2).

The epithelial basal lamina surrounding capillaries (capillary epithelial basal lamina) as well as flanking the mesangial regions (juxtamesangial epithelial basal lamina) was generally smooth and very uniform in thickness, except for rare spurs and hillocks projecting toward the epithelial side (Fig. 2). Occasional accordion-like wrinkling was observed in the juxtamesangial epithelial basal lamina. This was frequently associated with additional layers of smooth epithelial basal lamina apposed on the urinary side (Figs. 1–3). The inner, wrinkled layer frequently revealed an increase in electron-opacity, a focal to diffuse decrease in width, and fraying. The outer, smooth layer, which was always continuous with the adjacent capillary epithelial basal lamina, seemed to increase its thickness as the inner, wrinkled layer frayed and disappeared (Fig. 3). However, the juxtamesangial epithelial basal lamina was seen rarely with only one-half, or less, of its normal thickness, without being associated with an inner, wrinkled layer (Fig. 4).

The mesangial basal lamina, readily identified by extensive fenestration by cytoplasmic processes of mesangial cells (12) and less electron-opacity, was sharply

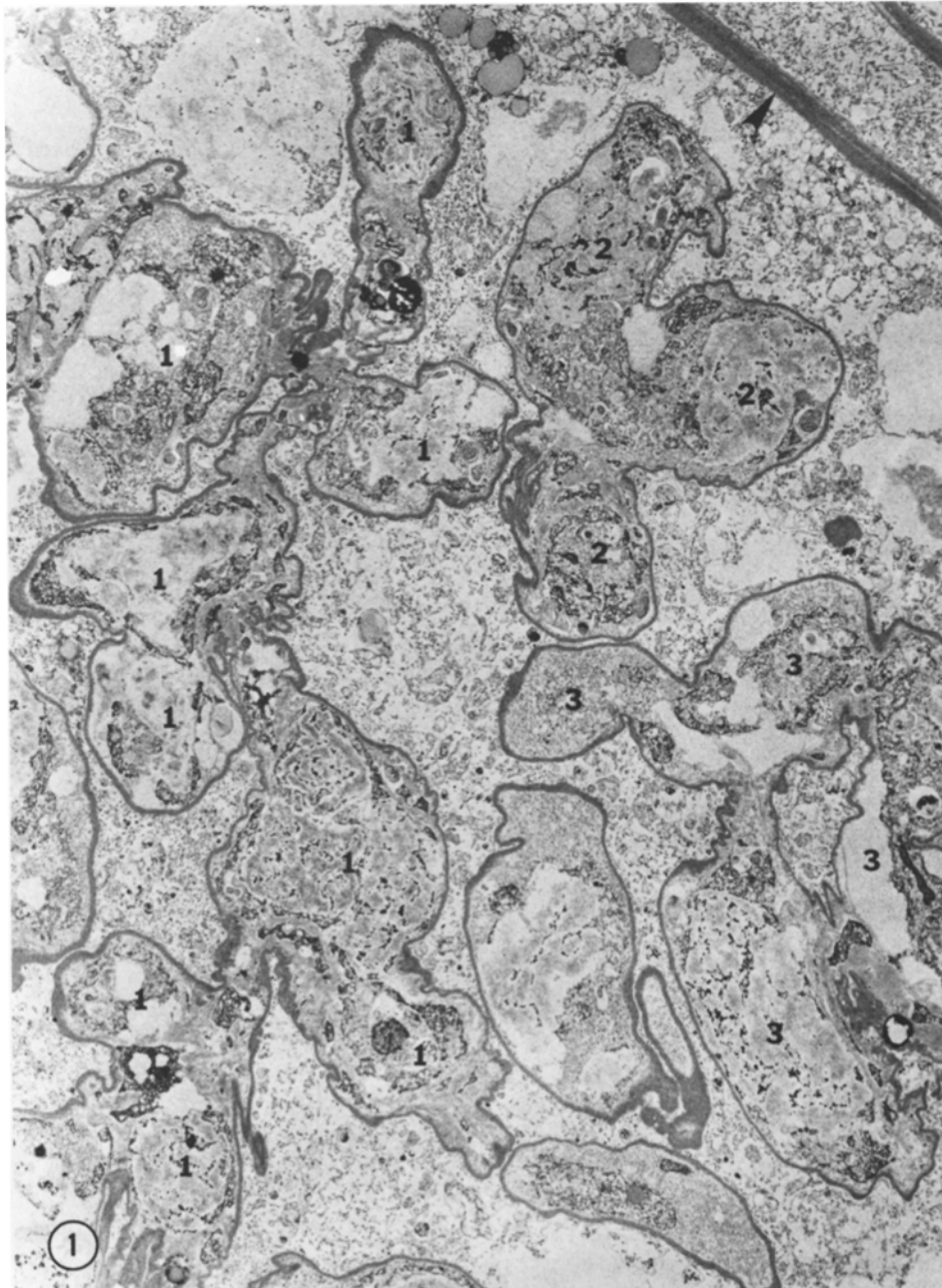


FIG. 1. A human renal glomerulus treated with guanidine solution reveals three capillary tufts (numbered 1, 2, and 3), each covered by a continuous layer of the epithelial basal lamina. The latter never completely surrounds the entire circumference of an individual capillary. A segment of the epithelial basal lamina of the Bowman's capsule (arrowhead) is shown at the right upper-hand corner. $\times 2,500$.

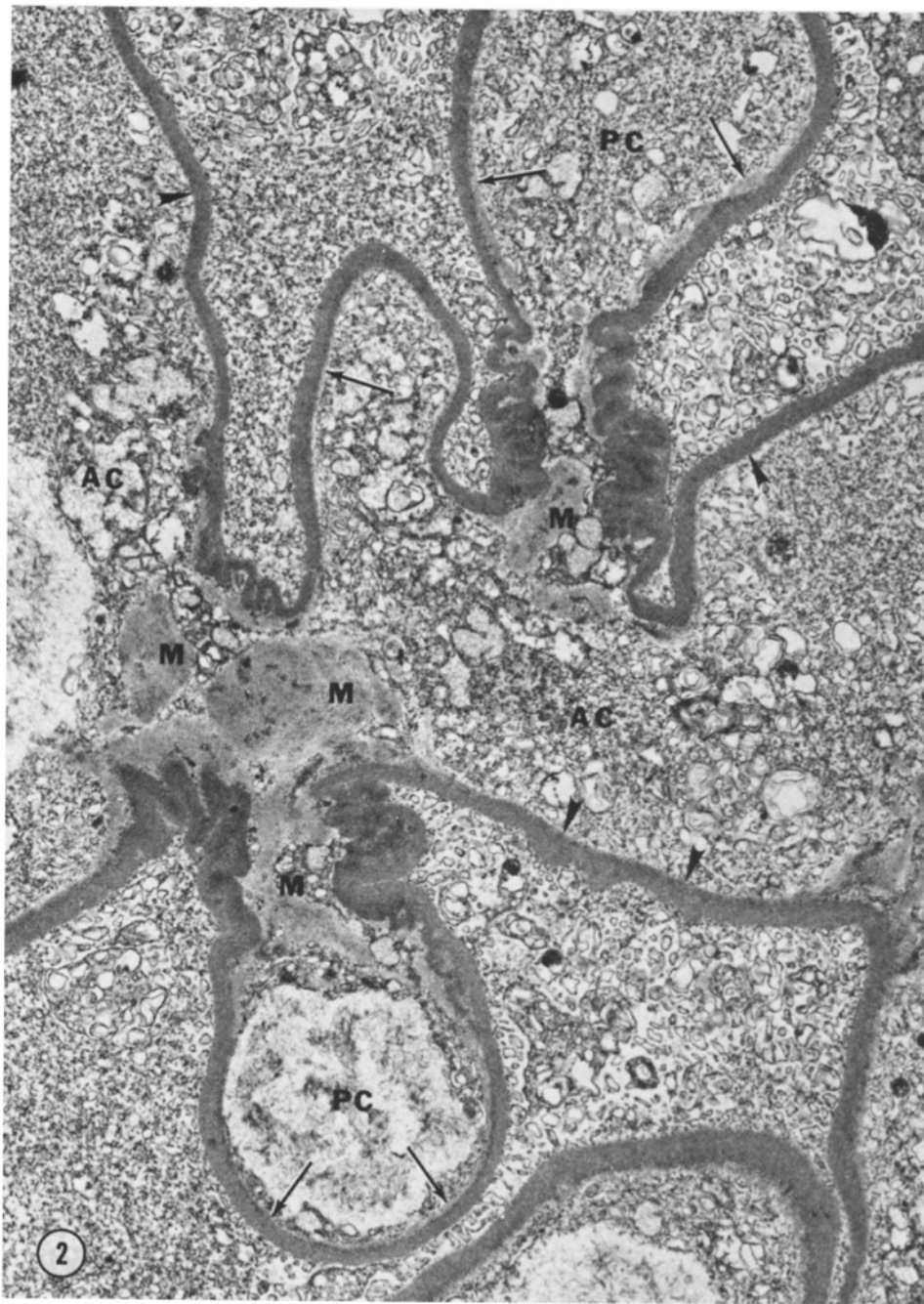


FIG. 2. A continuous layer of the epithelial basal lamina folds into loops of capillaries in a human renal glomerulus. The partition of the vascular space into individual capillary lumens is accomplished by the mesangial basal lamina (M), which has less electron opacity than the epithelial basal lamina after guanidine treatment. A thin layer of electron-lucent endothelial basal lamina (arrows), apposing on the capillary side of the epithelial basal lamina, surrounds the lumens of capillaries and is continuous with the mesangial basal lamina at the capillary waist. The endothelial basal lamina becomes extremely attenuated and undetectable focally (arrow-heads). Accordian-like wrinkling of juxtamesangial epithelial basal lamina is seen in two peripheral capillaries (PC), with additional layers of smooth epithelial basal lamina apposed on the urinary side. AC, axial capillary. $\times 8,250$.

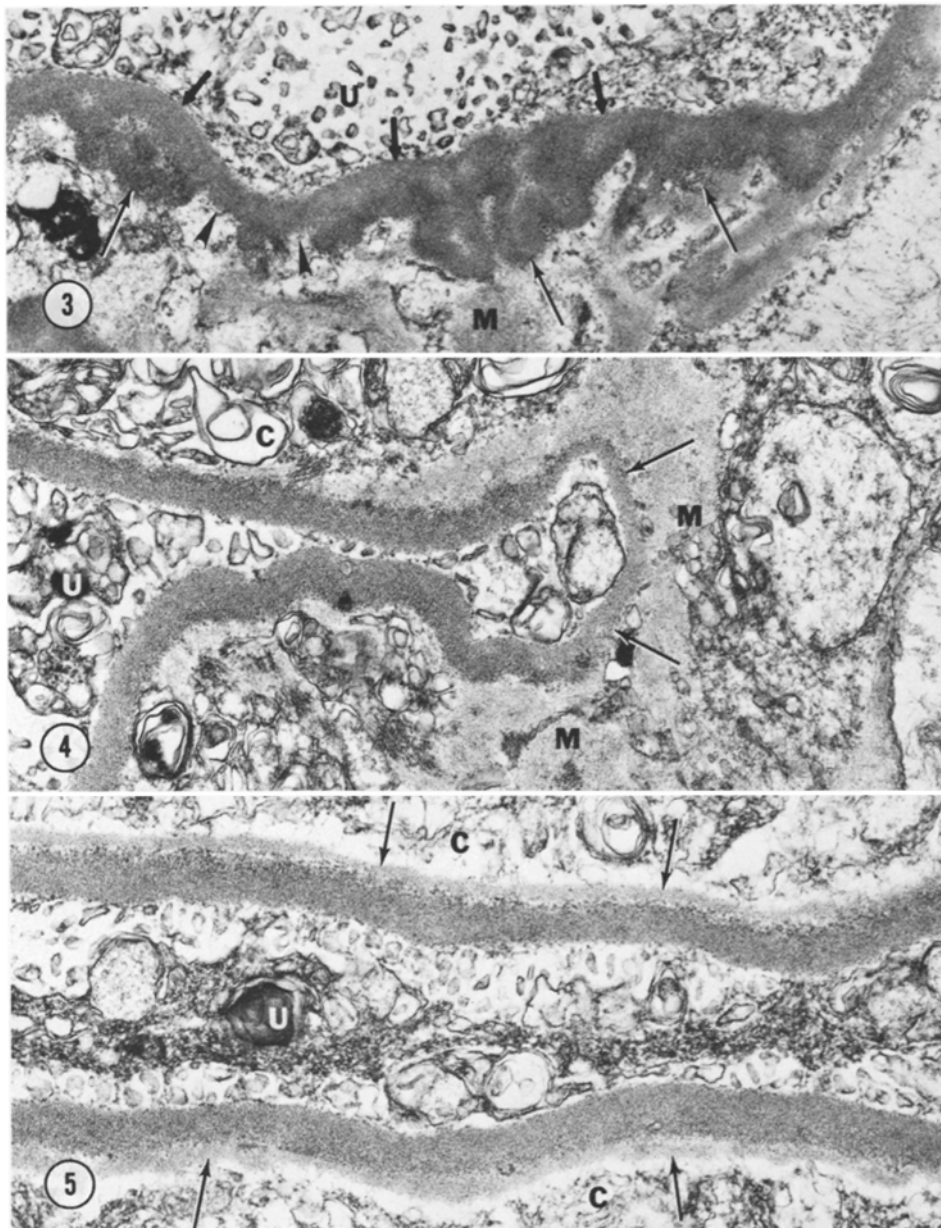


FIG. 3. A segment of juxtamesangial epithelial basal lamina reveals an inner, wrinkled layer (long arrows) and an outer, smooth layer (short arrows). The former discloses an increased electron opacity bordering the mesangial basal lamina (M) and is focally discontinuous (arrowheads). $\times 16,400$.

FIG. 4. A juxtamesangial epithelial basal lamina contains a thin segment (arrow). Its width decreases to one-fourth of the thickness of the adjacent capillary epithelial basal lamina. M, Mesangium. $\times 21,500$.

FIG. 5. Composite epithelial and endothelial basal laminae of two glomerular capillaries. The predominant element is the epithelial basal lamina on the urinary (U) side, which is apposed on the capillary (C) side by a thin layer of electron-lucent endothelial basal lamina (arrows). The line of demarcation between two components is always smooth and distinct. $\times 20,800$.

demarcated from the juxtamesangial epithelial basal lamina (Figs. 1-3). It was continuous with a thin layer of endothelial basal lamina of identical electron opacity, which surrounded the capillary lumens and closely apposed to the capillary epithelial basal lamina (Figs. 2 and 5). The endothelial basal lamina had its greatest thickness at the capillary waist and tended to become attenuated toward the periphery of capillaries, where it was frequently undetectable (Fig. 2). The line of demarcation between epithelial and endothelial basal laminae was always smooth and distinct (Fig. 5).

Discussion

The glomerular basal lamina is a bipolymer composed of collagenous and noncollagenous proteins (7) covalently cross-linked with disulfide bonds (15) and bonds derived from oxidative products of lysyl and hydroxylysyl residues (16). The extensive covalent cross-links render the basal lamina insoluble in various chaotropic agents (15), such as guanidine, while other cellular elements become disintegrated.

Glomerular Basal Lamina Heterogeneity. Previous studies of the glomerular basal lamina scaffold with conventional electron microscopy have suggested that the basal lamina surrounding the mesangial cells is different from the capillary basal lamina in that it is less compact and contains fibrils of 10 nm in width (12, 17). There also have been suggestions that the mesangial basal lamina may extend into peripheral capillary loops (18). Because of the technical limitations of conventional electron microscopy, clear distinction between the mesangial-endothelial and the epithelial basal lamina is extremely difficult. The current studies employing the guanidine technique have successfully identified two classes of basal laminae associated with three glomerular cell-types. The epithelial basal lamina is relatively electron-opaque, in contrast to the electron-lucent basal laminae of endothelial and mesangial cells. The latter two cell-types share a continuous basal lamina scaffold of identical electron lucency, presumably due to their common mesenchymal-vascular derivation (*vide infra*). The line of demarcation between the epithelial basal lamina and endothelial-mesangial basal lamina is always distinct. The designated endothelial basal lamina most likely represents the inner stratum of the lamina densa in capillary walls, although its relation to the lamina rara interna is unclear. No similar electron-lucent layers appear on the epithelial side where the lamina rara externa occurs.

Chemical studies of the glomerular basal lamina have also revealed heterogeneity of its constituent collagenous glycoproteins (19). Isolated human glomerular epithelial cells in cell culture have also been shown to synthesize collagenous proteins distinct from those synthesized by isolated mesangial cells (20, 21). These observations, together with kinetic studies demonstrating fast and slow turnover components of glomerular basal lamina (22), are in concordance with the glomerular basal lamina heterogeneity observed in the current studies.

Conservation of Histogenetic Schema in Adult Glomeruli. The spatial relationship of epithelial and endothelial-mesangial basal laminae disclosed in this study indicates a remarkable conservation in adult glomeruli of histogenetic schema observed during embryogenesis of glomeruli. The glomerulus is formed by invagination of an independent vasoformative mesenchymal cell mass into the lower end of the S-shaped metanephric vesicle. Initially, the glomerular visceral epithelium and its basal lamina are separated from the vascular tuft by an interstitial space. As glomeruli develop, the

interstitial space is obliterated and the epithelial and vascular basal laminas become fused (23–25). Despite undergoing great distortion from a smooth metanephric vesicle to an irregular plane covering a capillary-mesangial complex, the epithelial basal lamina remains as a single, continuous layer in adult glomeruli. It covers the glomerular capillary tufts as the serosa invests the intestine and mesentery. It never completely surrounds capillaries. The endothelial and mesangial basal laminas also remain as a continuum, connected to the vascular pole of the glomerulus and interstitial space of the kidney. The endothelial basal lamina in adult glomeruli is incomplete as it is during embryogenesis (25) and appears as a vestigial structure in glomerular capillary walls.

Basal Lamina Heterogeneity and Glomerular Pathophysiology. The glomerular basal lamina scaffold is one of the major filtration barriers of glomerulus (17). One of the principal determinants of the permeability property in terms of size-sieving is the density of the constituent collagenous fibrillar proteins (26) and the degree of covalent cross-links, analogous to agarose- and polyacrylamide-gel systems commonly used for molecular sieving in analytical biochemistry. The fact that the epithelial basal lamina swells significantly less than the endothelial basal lamina suggests the presence of a higher degree of intermolecular cross-links in the former (13), which may render the epithelial basal lamina less permeable to macromolecules, thus making it a more effective filtration barrier.

There are permeability differences between glomerular and systemic capillary basal laminas (17). The size limit of permeant molecules is <10 nm for the former, and 50–70 nm for the latter (27–30). The unique epithelial origin of the glomerular capillary basal lamina may account for its relative impermeability to macromolecules compared with other capillary basal laminas, which are predominantly of endothelial origin.

The finding that the epithelial basal lamina never completely encircles the entire circumference of capillaries may also provide an explanation for the low permeability channel composed of continuous endothelial-mesangial basal lamina. The existence for this functional channel has been amply demonstrated by various tracer studies (12, 27, 30–34). The tracers are found in the subendothelial location, presumably within the endothelial basal lamina, and in the mesangial basal lamina. Eventually, they are disposed of in the mesangium and the vascular pole of the glomerulus.

In addition to the permeability difference, the epithelial basal lamina seems to be more resistant to degradation and destruction in various glomerular injury. Habu snake venom and freeze-thawing cause mesangial cell necrosis and lysis of the mesangial basal lamina, leading to the formation of large, blood-filled spaces (25, 35, 36). Similar findings have been observed in human mesangiolytic glomerulonephritis (37). The capillary ballooning is due to the fusion of two or more adjacent capillaries sharing the same layer of the epithelial basal lamina, as depicted in Figs. 1 and 2.

Although the endothelial basal lamina seems to be a vestigial structure in normal glomerular capillaries, there is indirect evidence suggesting active participation of endothelial cells in glomerular capillary basal lamina synthesis in experimental glomerular reaction to injury (38, 39). Because the endothelial basal lamina may be more permeable than the epithelial basal lamina, the changes in the structure and relative proportion of these two elements and the consequent permeability alteration in various glomerular diseases deserve further investigation with the guanidine technique.

Juxtamesangial Epithelial Basal Lamina. The changes observed in the juxtamesangial epithelial basal lamina suggest that it may be the site of the bulk removal and renewal of epithelial basal lamina. A composite sequence of events taking place in this region is postulated as follows: (a) a contraction of mesangial cells (17) causes accordian-like wrinkling of juxtamesangial epithelial basal lamina (Figs. 1 and 2); (b) a new layer of smooth epithelial basal lamina is deposited on the urinary side by the epithelial cell (Figs. 2 and 3); (c) the wrinkled inner layer is subsequently degraded, fragmented, and removed by mesangial cells as the newly synthesized epithelial basal lamina is deposited on the urinary side (Fig. 3). The observed increase in electron opacity of the wrinkled layer may be due either to clogging or to partial chemical degradation of its constituent macromolecules. Asynchrony of the removal and the new epithelial basal lamina synthesis may have left a relatively thin juxtamesangial epithelial basal lamina as shown in Fig. 4. Because the epithelial basal lamina is a continuous layer, it is possible that its entire circumference can be renewed by this mode of turnover (circumferential mode) by the stepwise shifting of the position of the mesangium in relation to the epithelial basal lamina. This mechanism suggests an interconversion between capillary and juxtamesangial epithelial basal lamina.

Based on experimental argyria, another mode of basal lamina turnover has been proposed by others (40, 41), which postulates the continuous synthesis and deposition of epithelial basal lamina on the urinary side of the capillary basal lamina and its removal from the endothelial side (transmural mode). Further investigations are necessary to assess the relative importance of these two modes of epithelial basal lamina turnover in unclogging the filtration barrier (17) and in glomerular capillary remodeling in response to injury.

Summary

Two classes of glomerular basal laminae are identified with a newly developed guanidine technique. The electron-opaque epithelial basal lamina is the most prominent element of the glomerular basal lamina scaffold. It is a continuous layer within each glomerulus, folding into capillary tufts and loops, but never completely encircling the entire circumference of each capillary, similar to the serosa covering the intestinal loop and mesentery. The vascular space so defined is further partitioned into individual capillary lumen by an electron-lucent mesangial basal lamina, that forms a meshwork continuous with the vascular pole of the glomerulus and extends peripherally to surround capillary lumens. The latter, designated endothelial basal lamina, is extremely attenuated and appears as a vestigial structure in glomerular capillary loops. Changes in juxtamesangial epithelial basal lamina indicate that it may be the site of the bulk removal and renewal of the epithelial basal lamina.

The unique epithelial origin of glomerular capillary basal lamina and its organization provide a structural basis for understanding the glomerular physiology gained by various tracer studies. The results also suggest that the guanidine technique may be a useful new approach to the analysis of basal lamina alterations in various glomerular diseases.

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