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The glomerular mesangium: capillary support function and its failure under experimental conditions

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Summary. We present a structural analysis of the ability of the biomechanical unit consisting of mesangium and glomerular basement membrane to maintain normal capillary architecture in the face of mechanical challenges due to high intraglomerular pressures. Capillary support function may be considered in terms of the stabilization of local form (development of wall tension against capillary dilation) and global form (centripetal fixation of capillary loops to maintain higher order form). The pathologic consequences of the loss of this support are illustrated by way of experimental models of mechanical mesangial failure. Such failure may express itself as mesangial widening, increased transmesangial macromolecule "traffic," ballooning of capillary segments, and unfolding of capillary loops. Mechanisms are described by which these structural changes may lead to segmental glomerular sclerosis.

Key words: Kidney – Glomerulus – Mesangium – Mesangial failure – Electron microscopy – Animal models

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This paper describes the anatomic organization of the glomerular mesangium together with its geometric relationship to the glomerular basement membrane (GBM), the functional relevance of this

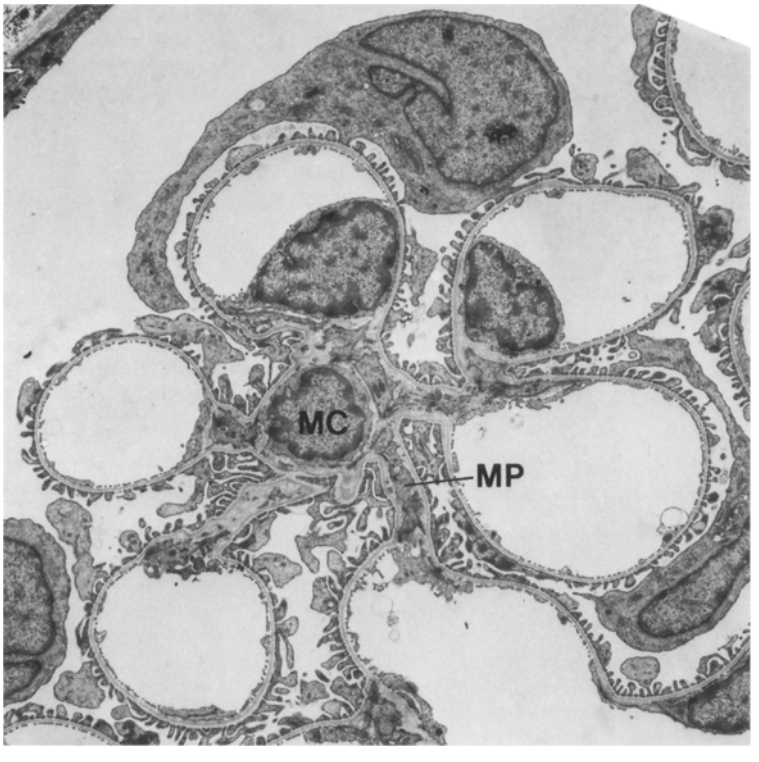


Fig. 1. Overview of part of a glomerular lobule (this and all other micrographs represent material from different experimental models in the rat, prepared by perfusion fixation and transmission electron microscopy methods as described in [21, 28]). The mesangium occupies a central position and is in contact with 6 surrounding capillaries. The glomerular basement membrane (GBM) and podocyte layers establish a single surface enveloping the mesangium (perimesangial portion) and the attached capillaries (peripheral portion). MC = mesangial cell body; MP = mesangial cell process. × 3400

system in supporting the glomerular capillaries, and some of the consequences of mesangial "failure."

General description of the glomerular tuft

The glomerular tuft (Fig. 1) comprises an anastomosing capillary network supported by a central mesangial stalk made up of mesangial cells and a dense fibrillar matrix [14, 18, 28]. The entire tuft is invested with a resilient basement membrane, the GBM. Outside the tuft but firmly attached to it by the GBM are the visceral epithelial cells (podocytes). If the capillaries are considered as tortuous endothelial cylinders wrapped about the mesangial stalk, then it must be kept in mind that along their entire length they are bordered by the mesangium over some part of their circumference. Since the GBM surrounds the combined endothelium-mesangium complex as a continuous sheet, no capillary segment is completely circumferentially surrounded by GBM.

Mesangial cells contain a contractile apparatus consisting of microfilament bundles found predominantly within the cell processes (Fig. 2). These bundles contain the usual contractile proteins such as actin, myosin, α-actinin, and tropomyosin (as demonstrated by immunocytochemistry [3]), and cell contractility has been demonstrated in vitro [14]. The mesangial cells or their processes insert (either directly or via extracellular fibrils) into the GBM at the "mesangial angles" [28], those points where the GBM deflects from a purely pericapillary course to envelop the mesangium. Within the axial mesangium, mesangial cell processes have multiple GBM attachments. Thus, both at the mesangial angles and within the axial mesangium, the GBM is the effector site for mesangial cell contractility.

Normal mechanical function

An appreciation of the mechanical aspects of capillary tuft architecture is vital to an understanding of normal and deranged glomerular function [12]. For example, the hydrostatic pressure gradient across the glomerular capillary wall is of the order of 40 mmHg [4]. Such a high pressure underlies effective filtration in these capillaries, but at the same time presents a mechanical challenge which is evidently much greater than that faced by ordinary tissue capillaries, in which a transmural pressure difference of 5–15 mmHg exists.

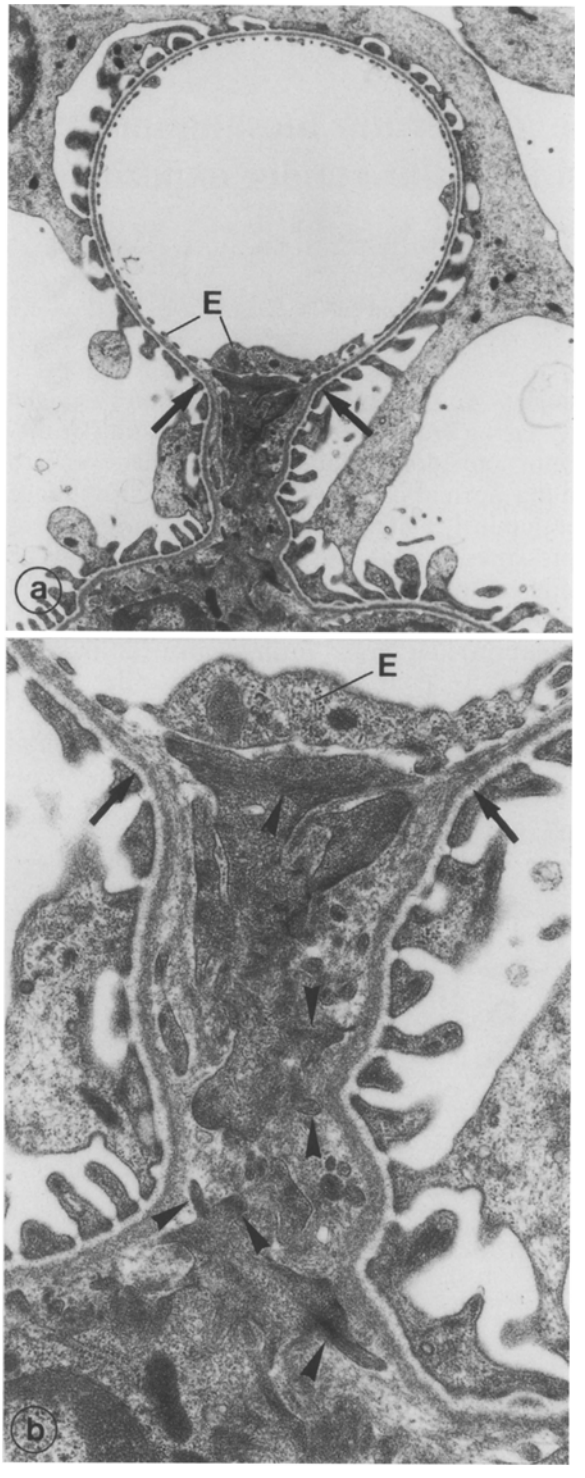


Fig. 2a, b. Overview of a glomerular capillary and its supporting mesangium. Most of the endothelial tube (*E*) is covered by the GBM; only a small portion contacts the mesangium. At the endothelium-mesangium interface, no basement membrane is developed. The mesangial cell gives rise to many cell processes which are filled with microfilament bundles (*arrowheads*) and attach to the GBM at the mesangial angles (*arrows*) as well as at sites on the perimesangial GBM. **a** × 6800; **b** × 18800

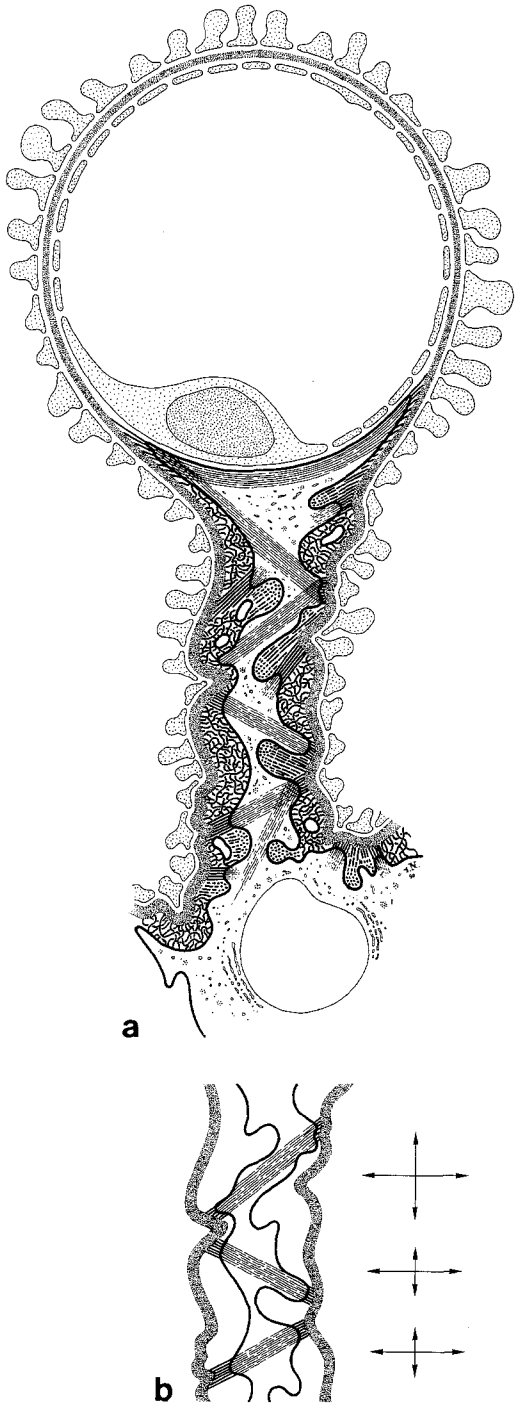


Fig. 3a, b. Schematic of a glomerular capillary and its supporting mesangium. At the mesangial angles, the GBM (shown in dark gray) deviates from a pericapillary course and covers the mesangium. The mesangial cell (only partly shown) and its processes are delineated by a *solid line*. Inside the mesangial cell process, the microfilament bundles are shown as *thin lines*; microfibrils of the mesangial matrix are depicted as *thicker lines*. The mesangium can be subdivided into axial (central) and juxtacapillary regions. Opposing parts of the GBM are in general connected by contractile filament bundles within the mesangial cell processes; these attach to the GBM either directly or by the interposition of extracellular microfibrils. These cross-bridges are especially prominent in the juxtacapillary region. In the axial mesangial region, the cross-bridges represent a system of generally obliquely running filament bundles. As shown in the lower schematic, which represents part of the central axial region from the upper schematic, these bundles together with the GBM establish a force-transducing system which can be conceptually separated into axial (vertical) and transaxial (horizontal) portions (directions of force transduction are indicated by *arrows*). Redrawn from [11]

Local stabilization of form

Such pressure gradients naturally give rise to expansile forces which have to be counteracted by the development of inwardly directed forces. In the case of the peripheral capillary wall these inward forces are generated via *wall tension*. The only structure in the peripheral capillary wall which seems capable of developing significant tension is the GBM [19], with its tough network of collagen fibrils [33]. The parts of the capillary abutting the mesangial stalk are not invested with a GBM (which instead "turns outward" to envelop the axial mesangial region), and the opposing mesangial angles are mechanically coupled by mesangial cell processes (Fig. 3).

In addition, given the virtual continuity of the capillary lumen and mesangium, which are separated by only a thin fenestrated endothelium over much of their interface, it is likely that the hydrostatic pressure within the mesangium (P_M) is approximately equal to the intracapillary pressure (P_{GC}). Thus, a pressure gradient of about 40 mmHg probably also exists across the perimesangial GBM, which will tend to expand the axial mesangial space unless counterbalanced by inwardly directed forces.

The mechanical contribution of the contractile apparatus of the mesangial cells to the development of stabilizing local counterforces is different in the peripheral capillary wall and the axial mesangial region. In the latter, the perimesangial GBM is stabilized in large part by the direct inward "pull" of mesangial cell processes (Fig. 3). The width of the mesangium is thus limited by the mechanical coupling of opposing segments of GBM.

The mechanical stabilization of the peripheral capillary wall is mediated by the development of wall tension, generated via extension of the elastic GBM. The relevant quantitative relationship is usually expressed in terms of the LaPlace relation for a cylinder, in symbols $\Delta p = \tau / r$. This principal states that wall tension τ is proportional to both the cylinder (here, capillary) radius r and the transmural hydrostatic pressure difference Δp. Equiva-

lently, the tension is inversely proportional to the wall curvature K, where $K = 1/r$, thus $\Delta p = \tau \cdot K$. This is illustrated by considering an infinitesimal wall element (Fig. 4a–c): The inwardly directed net force on this element (and thus the Δp which can be counterbalanced) clearly increases both with increased wall tension and with increased wall curvature.[1] The relatively minor acute changes in capillary radius which could be brought about by mesangial cell process shortening (certainly $< 10\%$, see Fig. 2a) limit the ability of the mesangium to normalize the capillary wall tension in response to increases in P_{GC}. Given this particular geometry, the LaPlace relation implies that at most a 10% increase in the capillary hydrostatic pressure gradient could be counterbalanced by a decreasing capillary radius before the wall tension would necessarily increase. The ability of the mesangial cell to alter the capillary radius on a longer time scale [7] could of course be based on mechanisms other than contractile shortening, e.g., an accelerated breakdown of GBM material (alternatively, a decrease in the rate of synthesis of GBM by the podocyte would have the same effect).

The high intramesangial hydrostatic pressures have another kind of mechanical consequence, a hydrodynamic one, as they might be expected to lead to significant filtration across the perimesangial GBM, which may be presumed to have a hydraulic permeability L_P comparable with that of the peripheral capillary wall. However, plasma proteins entering the mesangial matrix accumulate there, leading to a state analogous to a filtration pressure equilibrium in the glomerular capillary,

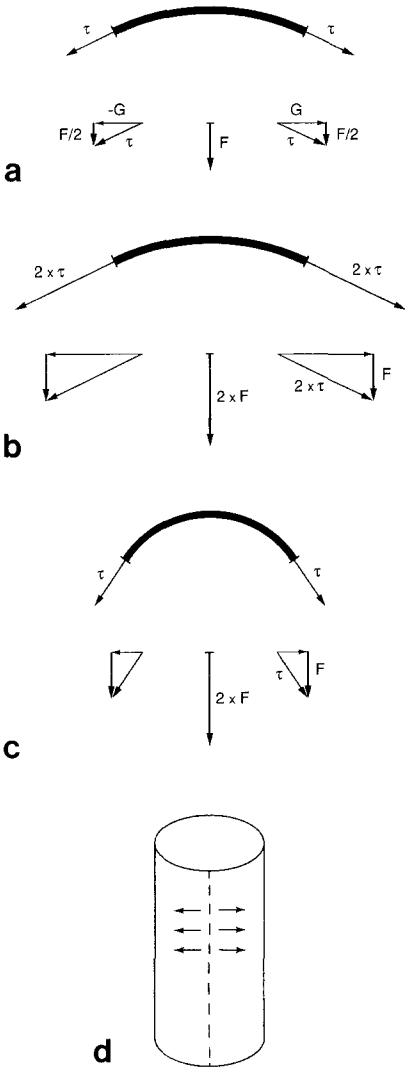


Fig. 4a–d. Schematic depiction of the forces acting on a small surface element of a cylinder (represented by a *thick black line*). **a** The baseline condition in which a net inward force of F suffices to counterbalance a given hydrostatic pressure gradient acting on the surface element. The membrane surface tension τ is represented as a force acting on the ends of the surface element. It is decomposed into tangential (G, -G) and inwardly directed (F/2) components, the vector sum of which is F. If the pressure gradient is doubled, the mechanical equilibrium can be maintained either by doubling the surface tension to $2 \cdot \tau$ (**b**) or by increasing the curvature of the element and thereby "directing" more of the tension-derived force inward (**c**). **d** The surface tensile forces act perpendicular to lines on the surface of the cylinder which are parallel to its long axis

in which the high transmural hydrostatic gradient favoring ultrafiltration is opposed by a significant oncotic pressure gradient. The transmesangial filtration rate is thus determined by (a) P_M (itself primarily a function of P_{GC}) and (b) the concentration of oncotically active mesangial proteins, which is a function of (1) the (slow) rate of filtration

[1] It must be remembered that wall tension is an intrinsically directional concept: It is the force per unit length acting across a line lying in the interfacial surface, in the case of the LaPlace relation, the force acting across a line perpendicular to the cylinder radius (Fig. 4d). In actual fact, a glomerular capillary is not a straight cylinder but rather a bent one, so any surface element has two nonzero *principal curvatures* K_1 and K_2 (where the principal curvatures are the maximum and minimum values which the directional curvature takes at that point on the surface). In this case, the relation takes the form [32]: $\Delta p = 2 \cdot \gamma \cdot \bar{K}$, where $\gamma =$ is the coefficient of surface tension, and $\bar{K} = {}^1/_2 (K_1 + K_2)$ is the mean surface curvature. Thus, the capillary radius alone does not suffice to determine wall tension.
In terms of understanding the effects of membrane forces on the actual structural elements of the capillary wall, wall tension is not a particularly useful concept. Its appropriate realm of application is soap bubbles and other phase boundary phenomena, where the source of the forces opposing "membrane" expansion is an interfacial free energy change, not the elastic properties of a membrane like the GBM. The actual force-bearing elements of the GBM (collagen fibrils of the lamina densa, for example) are subject to forces best reflected in the elastic wall stress, i.e., the force per wall cross-sectional area (essentially, the tension divided by wall thickness).

of smaller plasma proteins such as albumin across the perimesangial GBM and (2) the intramesangial degradation of large, nonfiltered plasma proteins. Under normal conditions, the rate of transmesangial fluid flux (which equals the rate of perimesangial ultrafiltration) is probably quite low. A hydrodynamic basis for the delivery of "filtration residues" from the peripheral capillary GBM to the mesangium seems unlikely.

Stabilization of capillary architecture

So far, we have considered how the stabilization of capillary form against local expansion is achieved through the generation of wall tension. We now consider the mechanism of global form stabilization, i.e., how mesangial cell contractility and GBM tensile strength together maintain and stabilize the shape and course of the glomerular capillaries. Specifically, we wish to know how this system maintains the intricately folded pattern of the GBM sack into which the capillaries are embedded.

To illustrate the problem quite prosaically, consider a common garden hose hanging bent under the force of gravity. When the hose is pressurized (whether or not water is flowing!) outward normal hydrostatic forces tend to dilate it, while an "excess" of forces on the outside of the bend tends to straighten the curve of the hose out. As described above, wall tension represents a restoring force against circumferential expansion. Mesangial cell contractility does not generate wall tension per se but provides an anchor against which wall tension can be developed by elastic forces in the GBM. In the hose, in contrast to the glomerular capillaries (vide infra), intrinsic wall forces do not oppose (but rather act in concert with) the hydrostatic forces, tending to straighten the hose out.

As depicted in Fig. 3, the form stabilizing system of the mesangium may be somewhat arbitrarily subdivided into two parts: the juxtacapillary system interconnecting the mesangial angles and the axial system consisting of short bridges between opposing parts of the GBM in the axial mesangial region. The juxtacapillary processes of mesangial cells form a fan-shaped series of clamps (Fig. 8a) along the mesangial-capillary interface. This system not only counteracts capillary dilation but also stabilizes the bent form of the capillary. By fixing the cylindrical form of the GBM sheath, the clamps bring into play the ability of an intrinsically bent elastic tube (i.e., one which has a longitudinally curved form in the *absence* of all forces) to resist straightening, since this would stretch the elastic material (here GBM) on the inside curvature and compress that along the outside curvature. The axial system also participates in the stabilization of the higher order form. This system consists of short bridges between opposing parts of the GBM, often appearing as obliquely running filament bundles. These bundles simultaneously stabilize against local mesangial widening as well as against "opening up" of the capillary loop. Although the components of this system do not necessarily take the shortest path in connecting opposing parts of the capillary loops, as a whole the system provides an indirect mechanical linkage between distant parts of the GBM which are thereby tethered centripetally, i.e., towards the central mesangial axis. This system will thus tend to minimize the development of longitudinal wall tension in the pericapillary GBM inasmuch as it fixes the ends of the capillary curve. Even this centripetal stabilization is effected by the cooperation of the GBM and the contractile apparatus in mesangial cells: Between insertions of mesangial filament bundles, the axial and transaxial counterforces are "transmitted" via GBM wall tension (Fig. 3b).

Dynamic mesangial function

The mechanical action of mesangial contractile elements on the GBM so far described is essentially static or, in analogy with the terminology of muscle physiology, one could say it is isometric (developing tension without inducing shortening). A dynamic role of the mesangium, for example, in acutely regulating the filtration surface area, has often been proposed [4] but seems unlikely. Since mesangial cell processes appear to be firmly anchored to the GBM at the mesangial angles, shortening of the intervening mesangial cell process would only bring the angles closer together, possibly compressing the mesangial-capillary interface, but leaving the peripheral capillary wall area (filtration surface area) unaltered. If mesangial contractility acutely alters the glomerular ultrafiltration coefficient K_f (as has often been concluded based on the effects of exogenous angiotensin II both on K_f and on mesangial cell contractility in vitro [2, 5]), it is therefore probably not by acutely changing filtration surface area. On the other hand, even quite modest changes in capillary radius ($\downarrow$ 5%, for example) could lead to substantial ($\approx \uparrow$ 25%) alterations in segmental flow resistance, possibly redirecting blood flow through the tuft and changing the total glomerular K_f by altering the proportion of loops in which filtration pressure equilibrium is attained [4, 25]. At present, a convincing mor-

phological basis for such a hypothetical regulatory system has not been described. In addition, any changes in capillary dimensions induced by angiotensin would have to be quite small, as they have not been observed in studies in which glomeruli are directly visualized in vivo [30]. In fact, most claims of dynamic tuft size changes attributable to in situ mesangial cell contraction are based on studies in isolated glomeruli [14], in which the mesangial cells are not under the same mechanical load as they are in vivo. It is likely that the increased "contractility" of mesangial cells induced by angiotensin II leads to little if any actual shortening, given the increases in capillary hydrostatic pressure which also occur under these circumstances. Thus, although a role for mesangial cells in acutely regulating K_f can by no means be excluded, a convincing mechanism is not at present known.

Mesangial mechanical failure

Just as the mechanical properties of the mesangial cell-GBM unit appear to underlie many aspects of normal glomerular function, failure of this unit may be involved in a number of pathophysiological phenomena, including the development of end-stage glomerulopathy. Basically, mechanical mesangial failure results from insufficiency of the contractile apparatus of mesangial cells or disruption of mesangial cell-GBM connections [29]. The concept of mesangiolysis discussed earlier by Morita and Churg [16] probably represents in this regard an extreme form of mesangial failure. The lesions seen in acute models of mesangial failure may appear later in "fixed" form in chronic models of glomerular injury.

As above, we can consider separately the local and global consequences of mesangial failure. Less severe (locally limited) mesangial failure may be expected to lead predominantly to mesangial expansion in the sense of a simple widening of the mesangial space. If mechanical failure is more widespread, the loss of capillary support function will lead to ballooning of capillary segments as well as unfolding of the capillary (Fig. 5). A heterogeneous pattern of lesions may also occur.

Several different models have been used to examine the acute effects of mesangial failure: the isolated perfused kidney [29], Habu snake venom nephropathy ([17]; unpublished observation from our laboratory), antithymocyte antiserum nephritis ([1]; unpublished observations from our laboratory). Many of the pathologic changes seen in chronic models of glomerulonephropathy (DOCA salt, experimental diabetes, chronic nephrotoxic serum nephritis) probably represent the results of repair mechanisms "aimed" at reestablishing structural stability in the tuft.

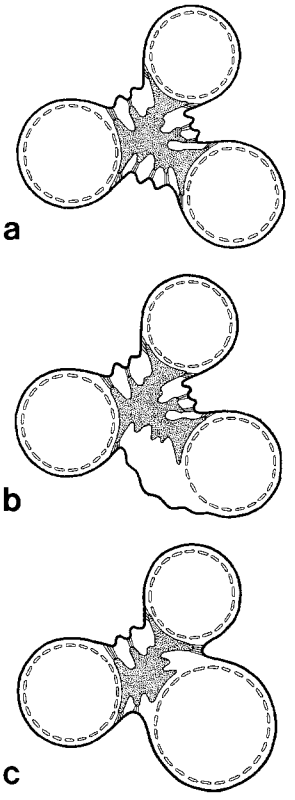


Fig. 5a–c. Schematic showing two possible scenarios of mesangial failure. **a** The normal situation with intact mesangial-GBM connections. Local disruption of these connections may lead to mesangial expansion (**b**) or capillary ballooning (**c**). Reproduced with permission from [13]

Olivetti and colleagues [22, 23] have demonstrated that relative mesangial failure can also be induced functionally, in their studies by means of acute angiotensin II-induced hypertension. Both an increase in mesangial accumulation of plasma proteins [22] and evidence of extensive capillary dilation [23] have been observed. These observations emphasize that mesangial insufficiency must always be understood relative to a particular level of mechanical challenge [29].

The mechanisms outlined below represent our understanding of some of the ways in which mesangial mechanical failure may escalate to pathologic structural changes, including segmental sclerosis. The question of the initial causes of mesangial failure is for the most part not addressed. The cause (*primum mobile*) is most clear in experimental models based on specific mesangial cell toxins (Habu snake venom, antithymocyte serum). In

most cases of human pathology, on the other hand, the culprit is likely to be *sustained capillary hypertension* (possibly acting synergistically with glomerular hypertrophy), leading to increased capillary wall tension and, via a purely mechanical overload, to mesangial contractile insufficiency.

Mesangial expansion

In the isolated perfused kidney (IPK) [29], disruption of the mesangial cell/GBM connections is followed by dissolution of the mesangial matrix and variable widening of the mesangial space. The damage increases with increasing perfusion pressure (and thus mechanical challenge to the tuft). Even early on, fluid-filled spaces are seen in the mesangial matrix, and with time the matrix becomes increasingly washed out, as more mesangial cell-GBM connections are broken. In the most severe stages, there is widespread dilation and distortion of the capillary loops (Figs. 6, 7a), whereas the mesangial cell viability is remarkably well preserved. The mechanisms responsible for mesangial failure are not fully clear but may include an increase in transmesangial filtration related to the dramatically altered hemodynamics found in this model.

A similar, but even more pronounced distortion of tuft architecture is seen in the antithymocyte antiserum model of mesangiolysis. The mesangial damage in this model is more severe than in the IPK in that it comprises virtually total lysis of the mesangium, including the mesangial cells.

Despite their appearance, structural changes such as those seen in the IPK or antithymocyte serum models are subject to repair [1]. In more limited forms of mesangial injury, small-scale disruptions of mesangial cell-GBM connections, leading to local outpocketings of the GBM, are later repaired by migration and/or reattachment of mesangial cell processes [11]. The loss of mesangial cell-GBM connections on a larger scale is a more catastrophic event. It seems likely that "architecture-preserving" repair is not possible under such circumstances and that such damage somehow triggers increased mesangial cell growth factor expression, leading to cell proliferation and increased matrix production [31]. The fixed lesion is the morphologic substrate of "mesangial expansion" in such chronic models (Fig. 7b). To the degree that mesangium-spanning contacts are reestablished between opposing parts of the GBM, mechanical stability is also reestablished. Fully normal function of the biomechanical unit (e.g., adaptive wall tension development, regulation of K_f, macromolecule clearance functions, etc.) may, however, be either temporarily or permanently lost. Exuberant repair of mesangial lesions probably plays a role in the development of the nodular segmental tuft lesion in diabetes [27]. It is unclear which factors are important in limiting the scope of mesangial "scarring" for repair rather than capillary obliteration.

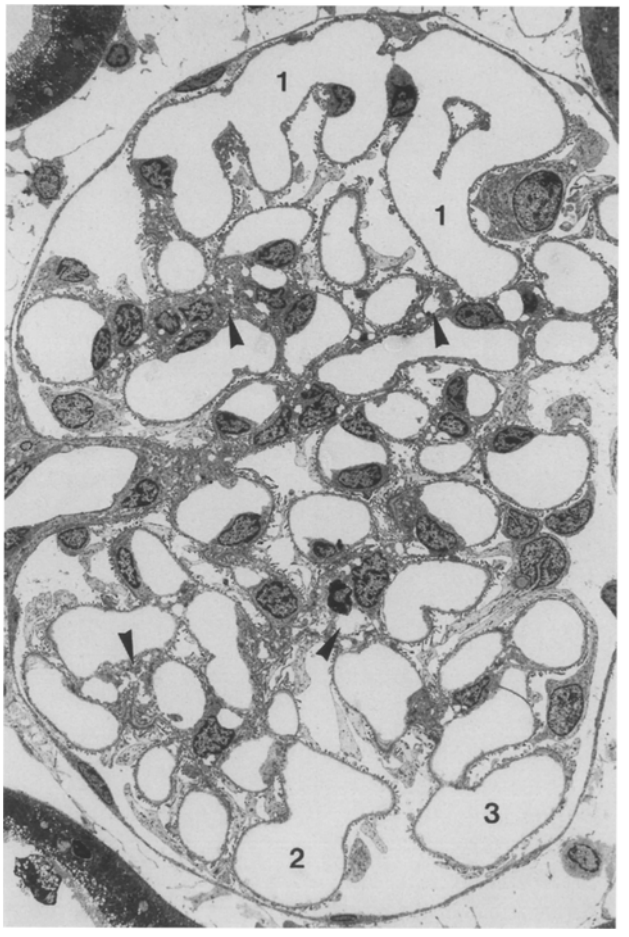
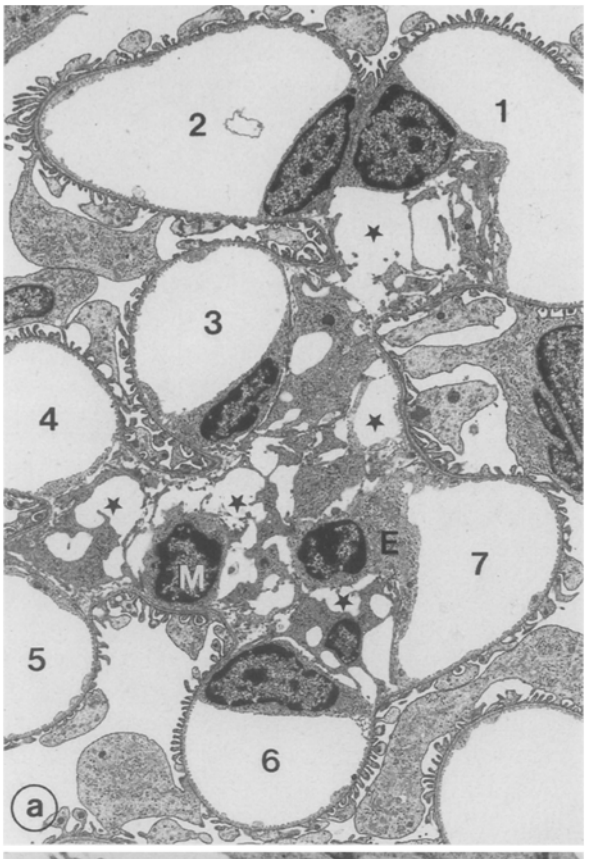
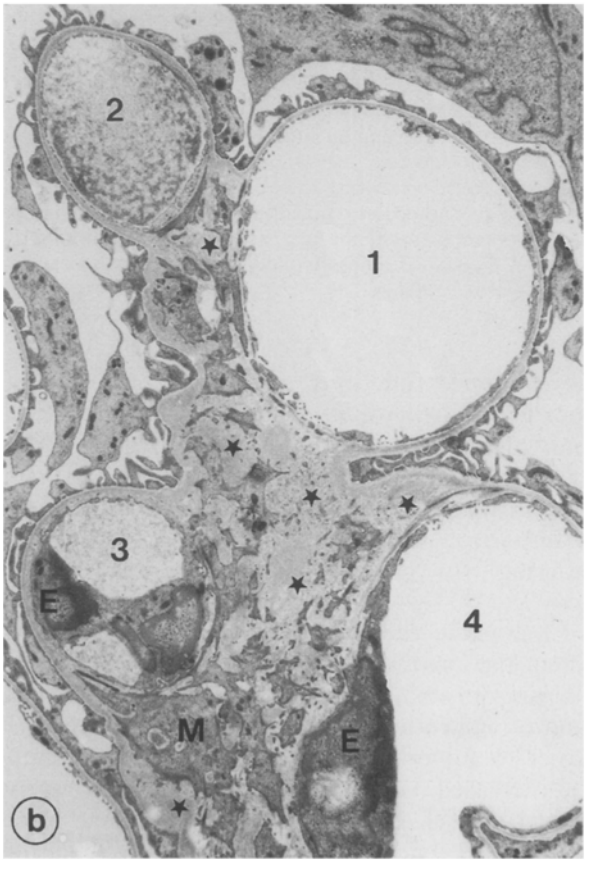
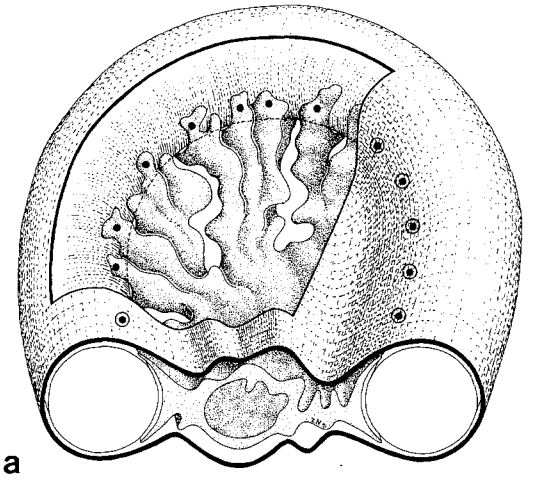


Fig. 6. Glomerular profile from an isolated perfused kidney (100 min perfusion time at 65 mmHg; for details, see [29]). The glomerular tuft contains several giant capillary profiles (*1–3*). At several sites (*arrowheads*), mesangial expansion is seen, including the appearance of fluid-filled spaces. Note the narrowing of Bowman's space; the peripheral capillary loops (most obvious in the dilated loops) actually touch Bowman's capsule in many places. ×920

Changes in the transmesangial fluid flux may explain the "pathologic" mesangial accumulation of plasma proteins seen in both experimental and human nephrotic syndrome. Mesangial regions subject to "overload" with plasma proteins may be at increased risk for the development of segmental sclerosis [8]. Such an accumulation has often been explained in terms suggesting that circulating







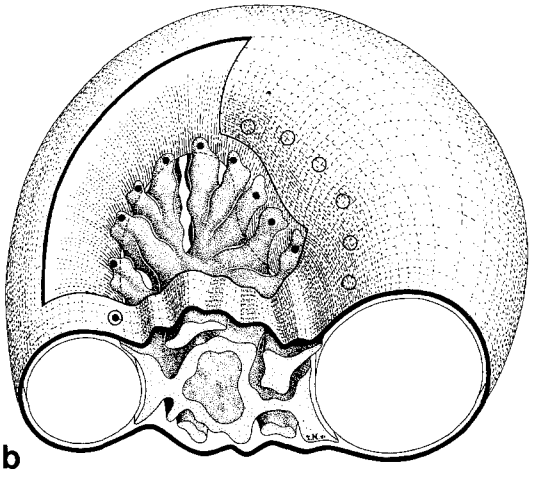


Fig. 8a, b. Schematic showing juxtacapillary clamp arrangement and its alteration in local mesangial failure. Part of the GBM is not drawn to allow a view of the clamps. Mesangial-GBM connections are represented symbolically as a row of snap fasteners. In a normal capillary loop (**a**), mesangial processes "snap" into the GBM envelop, stabilizing the local form against capillary dilation as well as maintaining the bent form of the entire loop. With progressive "unsnapping" of these connections (**b**), a bulge forms in the capillary, associated with mesangial retraction

Fig. 7a, b. Mesangial expansion in its acute form (**a**; isolated perfused kidney) and in its chronic form (**b**; DOCA salt model). **a** Peripheral subdivision of a glomerular lobule comprising seven capillary profiles arranged around the mesangial axis. The mesangium is greatly expanded. Within the mesangial area, confluent fluid-filled spaces are seen (*asterisks*). Many mesangial cell processes seem to have lost their connections to the GBM. **b** Mesangial axis associated with four capillary loops. The mesangium is greatly expanded. The expanded portions are densely filled with solid-looking extracellular material. Mesangial cell processes often appear to end blindly within the matrix. The capillary-mesangial interfaces are greatly broadened, and some capillaries (*3*) are even partially incorporated into the mesangium. **a** × 2900; **b** × 3400

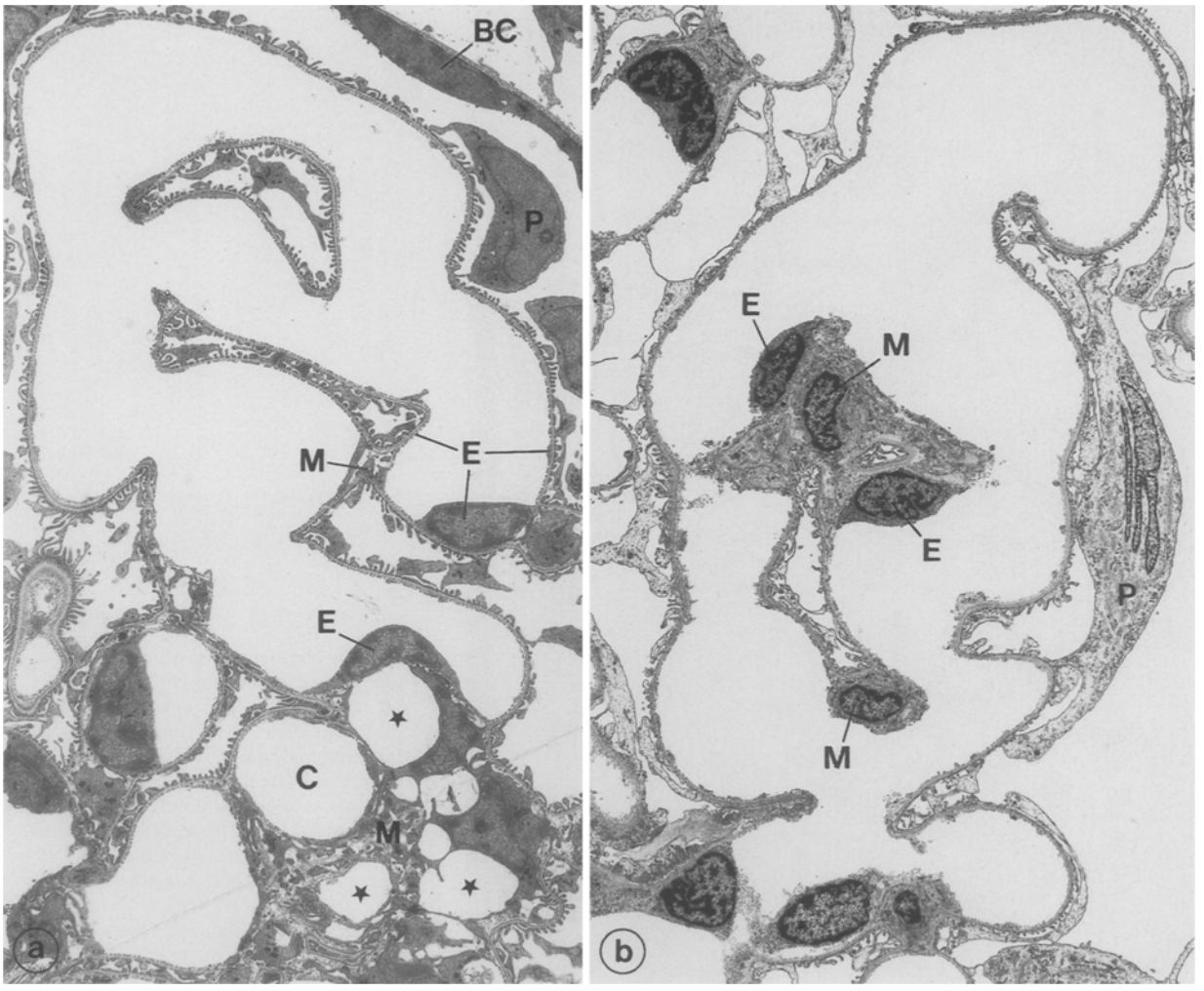


Fig. 9a, b. Capillary ballooning as it appears in an acute model (**a**; isolated perfused kidney) and in a chronic model (**b**; uninephrectomy in young rats) of mesangial failure. **a** A massively dilated and distorted capillary loop is seen. The mesangium (*M*) shows multiple fluid-filled spaces (*asterisks*). A small capillary (*C*) has been almost totally incorporated into the mesangium. The endothelium (*E*) and podocyte (*P*) layers are completely intact. **b** Massively dilated and distorted capillary loop. The mesangium has an unusual relation to the vascular channel. Note the extensive podocyte hypertrophy (*P*). BC = Bowman's capsule. **a** × 1800; **b** × 2000

plasma proteins gain access to the mesangial space on the basis of defects in glomerular permselectivity, as though under normal conditions there were a permselective barrier between the capillary lumen and the mesangium. Michael and colleagues [15], in contrast, recognized the importance of hemodynamic factors in this process. However, an increased glomerular capillary pressure by itself does not appear to provide a complete explanation of this phenomenon: P_{GC} is elevated both in models in which mesangial macromolecule accumulation occurs [22, 24] and in others in which accumulation is not seen [9]. In addition, accumulation also occurs during the "normotensive" phase of puromycin aminonucleoside nephrosis [8, 9]. We propose that alterations in any of the factors which affect transmesangial fluid flux (vide supra) may be responsible for mesangial macromolecule accumulation. Since filtration at the capillary-mesangial interface is nonselective [15], plasma proteins will be delivered to the mesangium in quantities proportional to their plasma concentrations and to the rate of transmesangial filtration. Thus, even if the absolute increase in transmesangial flux is low, the fractional increase in macromolecular delivery may be considerable. For those species of protein present in higher concentrations in the plasma and large enough to be only sparingly filtered at the GBM, immunofluorescently demonstrable accumulation will occur if the rate of delivery exceeds the maximal rate of intramesangial degradation. Thus, mesangial accumulation of im-

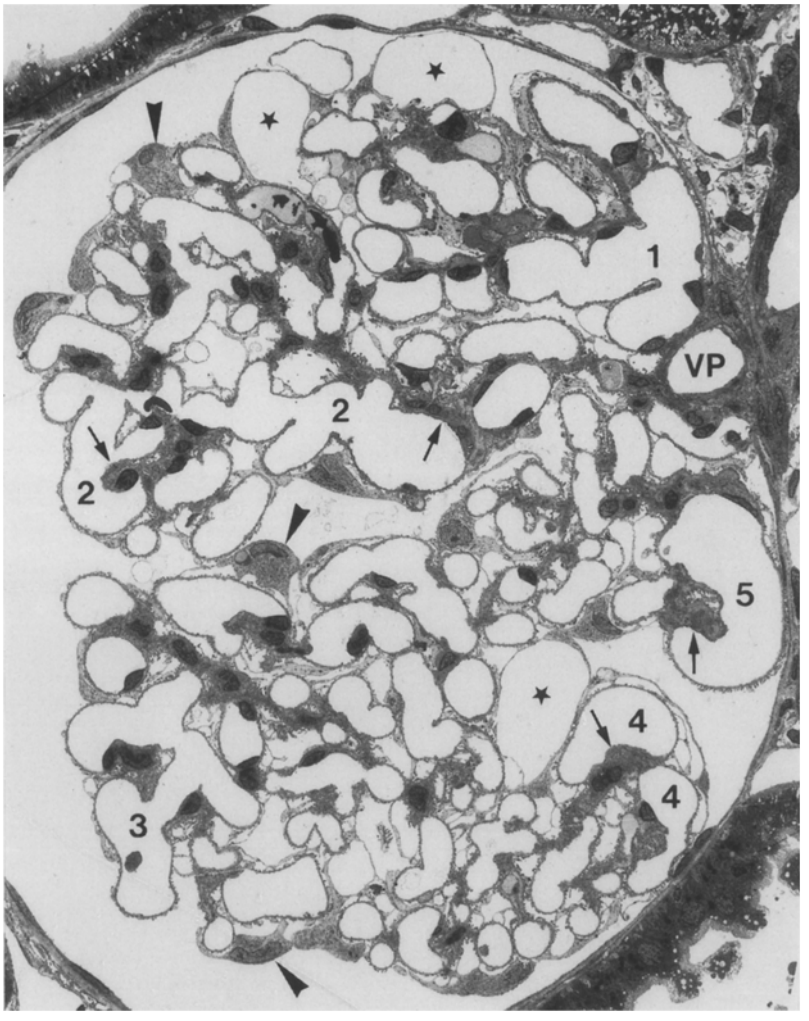


Fig. 10. Glomerular profile 24 weeks after uninephrectomy performed at 10 days of age. The glomerular tuft exhibits extensive distortion of the capillary architecture. At least five (*1–5*) abnormally shaped and dilated capillary channels are encountered; rearrangement of the mesangium is seen at several sites (*arrows*). Podocyte cell bodies are extensively hypertrophied (*arrowheads*); bleb formation (*asterisks*) is often encountered. Note the frequent apposition of tuft structures to Bowman's capsule. VP = vascular pole. × 500

munoglobulin M (IgM; ≈800 kDa, serum concentration ≈100 mg/dl), C_3 (186 kDa, 150 mg/dl), and IgG (160 kDa, 1200 mg/dl) in the human nephrotic syndrome may not necessarily be an immunologic phenomenon, but rather could simply reflect an increase in the rate of transmesangial fluid flow.

Glomerular capillary hypertension is clearly one factor which may increase the transmesangial fluid flow and has been shown to be associated with mesangial macromolecule accumulation [22, 24]. In addition, changes in the mesangial oncotic forces may also influence transmesangial filtration. Leakage of albumin across the perimesangial GBM – due to increased basement membrane macromolecule permeability – would result in an increased transmesangial filtration of plasma, reestablishing the filtration pressure equilibrium. This will lead to an increased mesangial delivery of larger proteins (such as IgM) which are essentially GBM-impermeant even in nephrosis, overloading the endogenous clearance systems and resulting in macromolecule accumulation.

Destruction of capillary architecture

A less localized mechanical failure of the mesangium may lead to a loss of capillary support function followed by extensive changes in the capillary pattern. The early changes of this kind can best be seen in the isolated perfused kidney model or in antithymocyte serum-induced mesangial failure; more advanced lesions, e.g., the development of microaneurysms, are best seen in Habu snake venom-induced mesangiolysis.

The changes in capillary pattern consist first in the ballooning of individual capillary loops, lesions which may develop into microaneurysms. To understand the mechanism of capillary ballooning, a three-dimensional approach is necessary (Fig. 8).

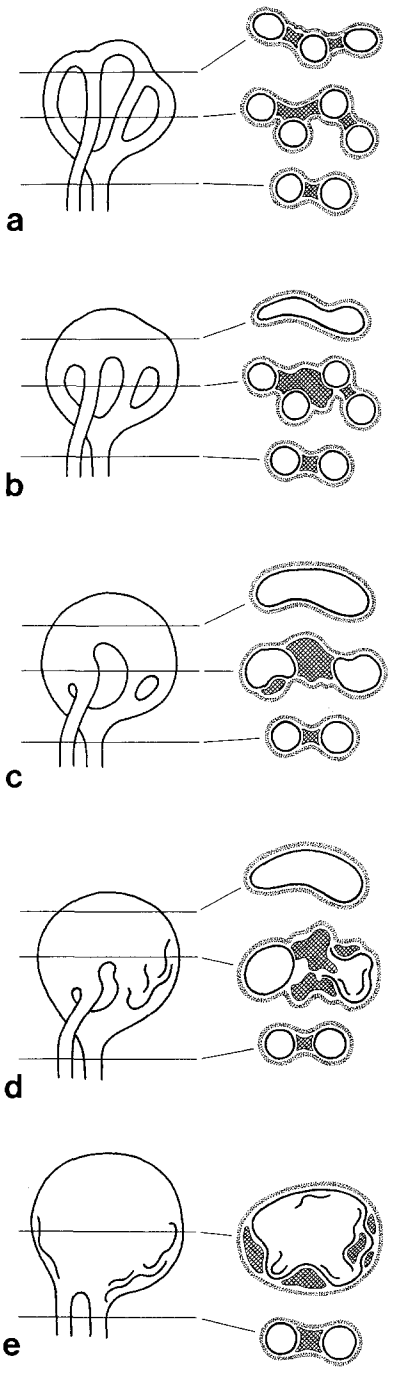


Fig. 11a–e. Schematic showing stages in the formation of a capillary microaneurysm. In the drawings to the *left*, only the endothelial tubes are shown for a subsegment of a lobule. In the corresponding drawings to the *right*, aross-sections through different levels of this capillary cluster are depicted for comparison. GBM is in *dark grey* and the mesangium is *cross-hatched*. **a** The normal capillary pattern. **b** The initial stages of capillary ballooning. The mesangium has retracted to a somewhat more central position, resulting in a dilated capillary loop. **c** A more advanced stage of capillary ballooning. The retracting mesangium has reached the confluence of two capillaries. A giant capillary channel is formed, of which the endothelial lining is still intact. In **d**, endothelial disruption at the capillary confluence leads to merging of the mesangial and capillary spaces, producing a blood cyst. **e** Further disruption of the endothelium leads to formation of a fully developed microaneurysm involving the entire capillary cluster. Remnants of the endothelium are found pressed together with mesangial tissue at the cyst wall

As outlined above and sketched in Fig. 8a, under normal circumstances the width of a capillary and its bent form are stabilized by the centripetal fixation of the GBM via the mesangial interconnection of opposing parts of the GBM. Among the centripetal fixations, the series of clamps along the mesangial-capillary interface is the most conspicuous. Contractile insufficiency or disruption of these connections over more than a very limited region will destabilize the form of the capillary loop, leading to local ballooning (Fig. 8b). As the mesangial damage proceeds, the process of ballooning will comprise additional parts of this and adjacent capillary loops, leading to a straightening out of the involved capillaries into abnormally shaped and dilated vascular channels [20, 21, 29]. Such channels are encountered in many models; examples are shown in Figs. 6, 9, and 10.

The formation of such dilated capillary loops occurs without obvious disruption of the endothelium (or of the epithelial layer). This process is accompanied by a rearrangement of the mesangium which is best seen in the IPK model (in the antithymocyte serum model, a much more widespread lysis of the mesangium seems to occur; unpublished observations). In the former model, the mesangium retracts centripetally and forms conspicuous agglomerations, which often protrude into the newly established vascular channels.

The ballooning of individual capillary loops may proceed to the formation of a microaneurysm, a process which can clearly be seen in Habu snake venom-induced mesangiolysis [17]. This process is sketched in Fig. 11. In the beginning (Fig. 11a–c), the retraction of the mesangium leads to capillary ballooning without endothelial disruption. When the process of mesangial retraction reaches a convergent capillary branching (a confluence of two capillaries), the endothelium will finally rupture, leading to a true coalescence of the capillary lumina and the mesangial space into a single blood cyst, i.e., a microaneurysm (Fig. 11d, e). Initial stages show small cysts, which may later coalesce into a microaneurysm comprising an entire glomerular lobule. The wall of such a microaneurysm consists of alternating endothelial (compressed capillaries) and mesangial layers (Figs. 11e, 12). The

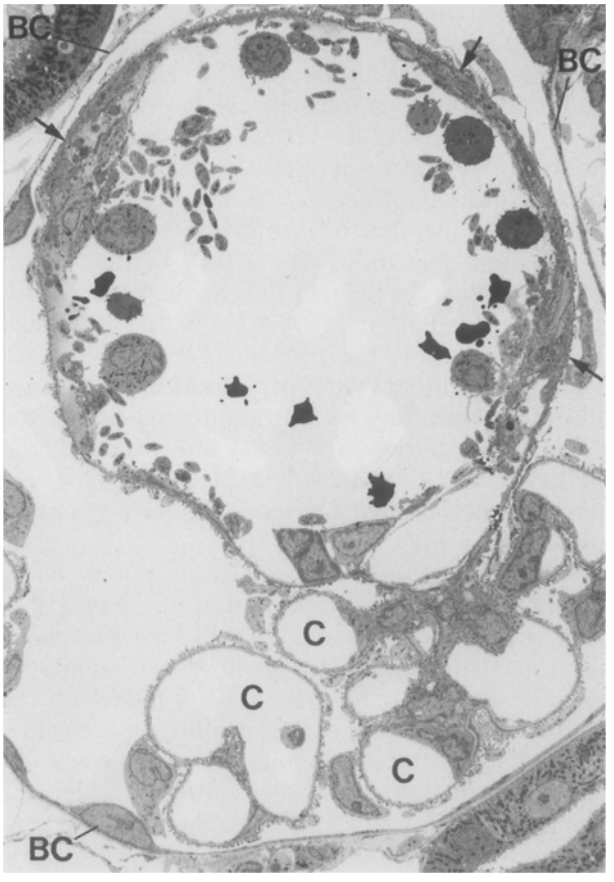


Fig. 12. Glomerular aneurysm from the Habu snake venom model. The aneurysm takes up a considerable part of the tuft. At several sites (*arrows*), the wall of the cyst consists of layers of endothelial and mesangial tissue in addition to the GBM. The capillaries (*C*) in the unaffected part of the tuft appear relatively normal. Within the cyst, blood cells and thrombocytes may be seen. BC = Bowman's capsule. ×1000

crucial development separating simple capillary ballooning from aneurysm formation is disruption of the endothelium.

In chronic models (uninephrectomy, nephrotoxic serum nephritis, Habu snake venom), both the process of capillary ballooning and that of microaneurysm formation may lead to segmental glomerulosclerosis; however, the pathways in the two cases appear to be quite different. In the latter, endothelial damage is the crucial event; in the former, epithelial damage seems to initiate the development of sclerosis.

We first consider the pathway via microaneurysms. The development of segmental glomerular sclerosis from a microaneurysm is straightforward [6]. Aggregated thrombocytes at the damaged endothelial-mesangial interface will initiate the formation of a thrombus, eventually occluding the lumen of the cyst. Organisation of this thrombus will lead to segmental sclerosis. On the other hand, in both experimental models and human pathology, microaneurysms are relatively rare events [26]. Therefore, this pathway would appear to be of only marginal importance in the development of chronic glomerulonephropathies. A possible exception is diabetic glomerulosclerosis [27], which probably differs in several respects from most other models.

In contrast, a pathway to segmental sclerosis starting with simple capillary ballooning appears to be of greater relevance in most experimental systems and possibly in human nephropathies as well. The following description is based on our studies in the remnant kidney after uninephrectomy in young rats [20, 21]. In this model the ballooning of capillary loops is a frequent event (Figs. 9b, 10). Any enlargement of a glomerular capillary (e.g., secondary to mesangial failure) challenges the adaptive potential of the podocytes. Podocytes have to maintain the urinary surface cover of all such loops. No doubt, these cells have a certain capacity to adapt to such challenges acutely, and they enter a phase of adaptive cell hypertrophy if the challenge is prolonged. However, podocytes appear to have little or no mitotic potential postnatally, which places ultimate limits on their adaptive capabilities.

The adaptive potential of the podocytes will reach its limit if they are subjected to multiple mechanical challenges at the same time, as may occur in any glomerulopathy associated with compensatory tuft hypertrophy. Compensatory tuft growth per se is frequently associated with considerable maladaptive changes in the podocytes: cell body attenuation, lengthening of primary processes, and formation of pseudocysts.

The additional challenge imposed by capillary ballooning will aggravate the situation in two respects. First, the affected podocyte may become so seriously overtaxed that localized detachment from the GBM occurs. Second, having lost its centripetal attachment, the affected tuft region will bulge toward Bowman's capsule, leading to apositions between the capsule and the damaged podocytes. Detachment of podocytes from the GBM at this site allows access of parietal cells of Bowman's capsule to the GBM, establishing a "beachhead" for the subsequent formation of an adhesion (Fig. 13).

In agreement with previous findings in nephrotoxic serum nephritis [10], we postulate that an adhesion represents the decisive nidus for the development of segmental sclerosis. Thus, local me-

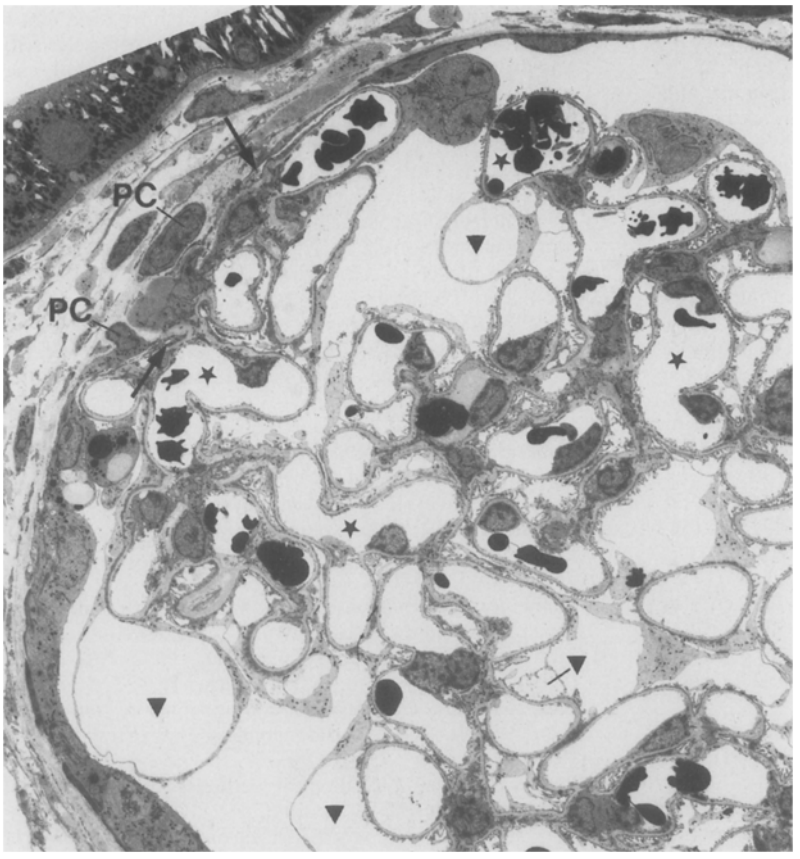


Fig. 13. A small tuft adhesion to Bowman's capsule (delineated by *two arrows*) in an early stage of development. Lesions within the adjoining tuft area include capillary dilation (*asterisks*) and podocyte bleb formation (*triangles*). However, neither capillary obsolescence nor sclerotic foci are seen. The actual attachment is brought about by parietal cells (*PC*) connected to the GBM. ×970

sangial failure associated with capillary ballooning and destabilization of the lobule architecture is a pivotal event, which by subjecting the podocytes to supraphysiologic mechanical stresses may eventually lead to tuft adhesions and finally segmental sclerosis.

In conclusion, the renal glomerulus is constantly exposed to high hydrostatic pressure gradients. The principal structure actively meeting this mechanical challenge is the mesangium. Loss of mesangial support leads to pathophysiologically significant changes in tuft structure, i.e., mesangial expansion, capillary ballooning, and the formation of microaneurysms. These (often localized) manifestations of mechanical failure represent starting points for at least three distinct pathways to segmental glomerular sclerosis.

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