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The structural relationship between mesangial cells and basement membrane of the renal glomerulus

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Summary. It has been shown by many studies that mesangial cell contraction exerts considerable influences on glomerular filtration dynamics. However, experimental findings about the geometrical changes within the glomerular tuft going along with mesangial cell contractions are lacking. This study analyzes the geometry of mesangial cells and their relationship to glomerular capillaries, especially to the glomerular basement membrane (GBM).

By applying a new staining technique of unosmicated specimens for TEM, the cellular outlines of glomerular cells (mesangial, endothelial and epithelial) and the distribution of extracellular matrices can be more easily studied than in conventionally osmicated specimens. It became obvious that mesangial cells and the GBM are extensively connected with each other, either by direct attachments or indirectly by microfibrils. These connections are especially prominent at mesangial angles, i.e. at sites where the GBM deviates from its pericapillary course and covers the mesangium. Thereby, the GBM is not only coupled to the mesangium but – via mesangial cell processes – also to the GBM at the opposing mesangial angle. It seems possible that contraction of mesangial cells can bring the GBM from opposing mesangial angles closer together. Therefore we conclude that the GBM and the contractile mesangial cells together establish a biomechanical unit capable of developing wall tension in glomerular capillaries and of changing the geometry of glomerular capillaries following mesangial contraction or relaxation.

Key words: Renal glomerulus – Mesangial cells – Mesangial matrix – Glomerular basement membrane – Microfibrils

Introduction

The glomerular capillaries are perfused at a high hydraulic pressure that exerts a distending force to the capillary wall. The wall structure of glomerular capillaries is unique; a specific concept of how the distending forces to the capillary wall are counterbalanced in this specific type of capillaries has so far not been developed. The problem, when compared with other capillaries, arises from the fact that neither the glomerular basement membrane (GBM) nor the cellular layer of podocytes encircle completely the glomerular capillaries. Both structures deviate from a circular course at the mesangial region. On their own they are therefore unable to balance the distending forces by developing wall tension. Endothelial cells establish a continuous circular layer that fully outlines the inner circumference of glomerular capillaries. These cells, however, have a highly attenuated and fenestrated cytoplasm which very unlikely has the strength to resist the transmural pressure difference.

The third cell type of the glomerular tuft, the mesangial cells are smooth muscle like contractile cells which occupy the axial region of a glomerular lobule. They have first been described by Zimmermann (1929); since the confirmation of their occurrence by ultrastructural techniques (Latta et al. 1960; Farquhar and Palade 1962) they have been in the focus of glomerular research. It is widely believed that they form a supporting frame work which maintains the structural integrity of the glomerular tuft including its capillaries. Moreover, mesangial cells possess receptors to vasoactive hormones such as angiotensin II (Sraer et al. 1974; Skorecki et al. 1983). It has been suggested that mesangial cell contraction underlies the change in glomerular filtration coefficient and hence glomerular filtration rate produced by a number of vasoactive substances (Ausiello et al. 1980; Kreisberg 1983; Foidart and Malieu 1986; Brenner et al. 1986). However, a biomechanical concept is lacking of how mesangial cells act on glomerular filtration dynamics. The precise geometry of mesangial cells is unknown and their structural relationships to glomerular capillaries are not fully understood.

In the present investigation we have studied the ultrastructural geometry of mesangial cells with special emphasis on the relationships to the GBM. For a better visualization of cellular outlines as well as of the intra- and extracellular fibrillar structures, a technique to stain unosmicated specimens for TEM (Sakai et al. 1986) was modified and employed in the present study.

Materials and methods

Female Wistar rats (Ivanovas, Kissleg; BW 120–180 g) were used. After anesthesia with Inactin (10 mg/100 g BW) the abdominal cavity was opened and the kidneys were per-

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Table 1. Dehydration protocol

Aceton	Temperature	Time
30%	+ 5° C	30 min
50%	0° C	60 min
70%	−20° C	60 min
90%	−30° C	60 min
100%	−30° C	60 min
100%	−30° C	60 min
100%	−30° C	60 min

fused via the abdominal aorta as described previously (Kaissling and Kriz 1982). Briefly, via a retrograde canula perfusion fixation was carried out at a pressure of 220 mm Hg for 3 min without prior flushing of the vasculature. The fixative contained 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) supplemented with 0.08% $CaCl_2$, 0.5 g/l picric acid and 30 g/l hydroxyethylcellulose. In some animals the kidneys were flushed prior to fixation. As flushing solution the cardioplegic solution HTK (Histidine-Tryptophan-Ketoglutarate) was used (Bretschneider 1980; Schnabel et al. 1985; Kallerhoff et al. 1986), which contained 15 mM NaCl, 9 mM KCl, 4 mM $MgCl_2$, 180 mM histidine, 18 mM histidine-HCl, 1 mM K-α-ketoglutarate, 2 mM tryptophan, and 30 mM mannitol (pH 7.3; osmolarity 310 mosm/l). Flushing was carried out at 6° C at a pressure of 150 mm Hg for 30 s followed by perfusion with the same fixative as mentioned above at a pressure of 150 mm Hg for 3 min. After perfusion the kidneys were removed immediately, cut into slices, and immersed in the same fixative for at least 12 h. The tissue was washed thoroughly in cacodylate buffer.

The slices were not osmicated, but stained en bloc either before or after dehydration (pre- and post-dehydration staining). Tissue slices for pre-dehydration staining were immersed in the dark in a solution of 1% tannic acid in 0.1 M maleate buffer for 3 h at room temperature and washed in the same buffer for another 2 h, followed by staining in a 1% uranyl acetate solution in the same buffer in the dark at 4° C. Some of the slices were treated before staining with a 5% DMSO solution (dimethylsulfoxide) in 0.1 M maleate buffer. Dehydration of the tissue slices (stained and unstained) was carried out in graded series of acetone at gradually decreasing temperatures (Carlemalm et al. 1982) according to a schedule shown in Table 1. After dehydration, so far unstained slices were stained in acetone at −30° C containing 1% tannic acid for 16 h, followed by 1% uranyl acetate for 8 h. Immersion into a 1/1 mixture of propylene oxide and Epon was still done at −30° C. Afterwards the temperature was allowed to rise and the final embedding into Epon 812 was done by standard procedures. Some additional tissue slices were routinely postfixed with 1% OsO_4, dehydrated in ethanol and embedded in Epon 812.

Ultrathin sections (gray to silver) were cut with a diamond knife, stained with uranyl acetate and lead citrate and then observed in a Philips 301 electron microscope.

Results

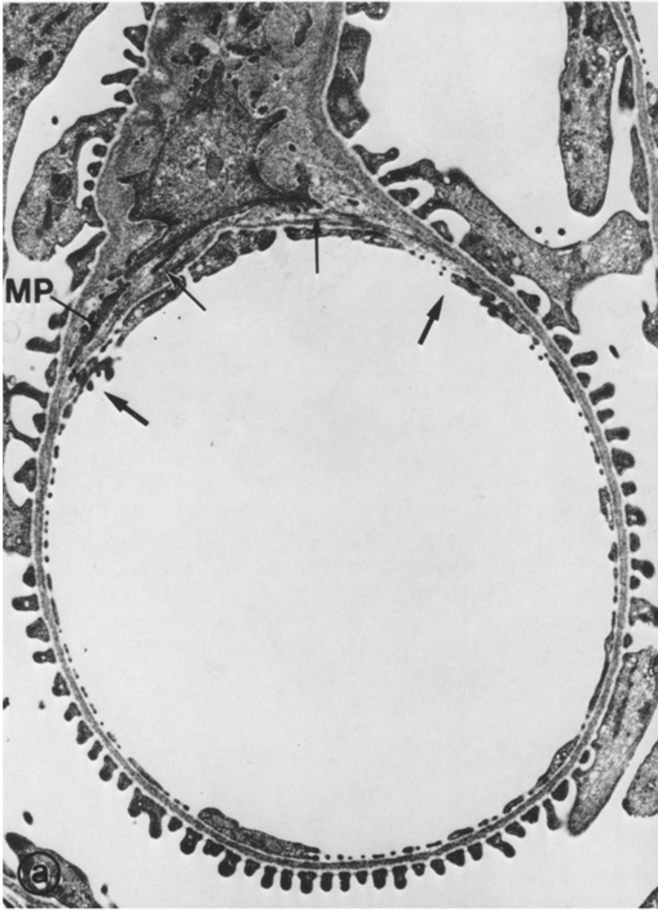
The ultrastructure of cells and extracellular matrices was found to be better preserved in our non-osmicated, cold dehydrated specimens than in conventionally prepared osmicated samples. First, all cell membranes exhibit a more distinct appearance; as has been shown in a previous study (Sakai et al. 1986), cell membranes were found to be thicker than in usual osmicated specimens and are prominent by a thick clear middle zone. Therefore, the course of cell membranes could be much more easily traced which was especially helpful with regard to the complicated geometry of mesangial cell processes. Second, all cytoskeletal elements, such as microfilaments, intermediate filaments and microtubules were well preserved. Third and most important for this study, extracellular matrices, extracellular fibrils and filaments both in the GBM and in the mesangial matrix showed a more definite appearance than in osmicated specimens.

On the other hand, there are disadvantages of this technique. After predehydration staining the tissue is often inhomogeneously stained. As is known from other studies using tannic acid (Maupin and Pollard 1983; Goldman et al. 1979; Simionescu and Simionescu 1976; LaFountain et al. 1977) unstained cells are frequently found intermingled with those that are darkly stained. After pretreatment with DMSO a more homogeneous staining pattern was achieved. Moreover, cytoplasmic and mitochondrial membranes are often poorly and inconsistantly stained. Fortunately, for the purpose of this study these disadvantages are of minor relevance. A more detailed description of the scope of this modified tannic acid technique will be given elsewhere.

The structure of a glomerular lobule

A glomerular tuft is composed of several lobules. Each lobule is established by an axially located mesangium around which the glomerular capillaries ramify. A small part of the outer circumference of each capillary is in touch with the mesangium (Figs. 1, 2). The glomerular capillaries are made up of an endothelial tube. The GBM and the podocytes are generally interpreted as additional layers of the capillary wall but they do not completely encircle the endothelial tube. The GBM and the layer of podocytes constitute a common surface cover wrapping together the capillaries and the mesangium. Therefore, in each of these components (capillary wall, GBM, podocytes and mesangium) two subdivisions must be considered. The capillary wall consists of an *urinary portion* which bulges into Bowman's space and is covered by the GBM and podocytes and a much

Fig. 1. Profiles of glomerular capillaries showing various appearances of mesangial angles (indicated by *thick arrows*). In all four profiles the juxtamesangial portion of the endothelium is made up of either peripheral parts of the cell body cytoplasm or of fenestrated cytoplasmic parts. A difference in thickness of the pericapillary and the perimesangial part of the GBM is apparent in all four profiles; the latter is partially wrinkled. In **a**, **c** and **d**, tongue-like processes of mesangial cells (*MP*) run towards the GBM at mesangial angles; in **b**, profiles of mesangial microprocesses (*MP*) are encountered. Note the loose connection between the juxtamesangial portion of the endothelium and the underlying mesangial cell process (in **b**, **c**, and **d**); in **a** tangentially running fibrillar structures (*thin arrows*) are found beneath the endothelium, in **d** those fibrillar structures extend far into the space between the endothelium and the GBM (*thin arrows*). **a–c** pre-dehydration, **d** post-dehydration staining. **a** × ∼8500, **b** × ∼10500, **c** × ∼8000, **d** × ∼10500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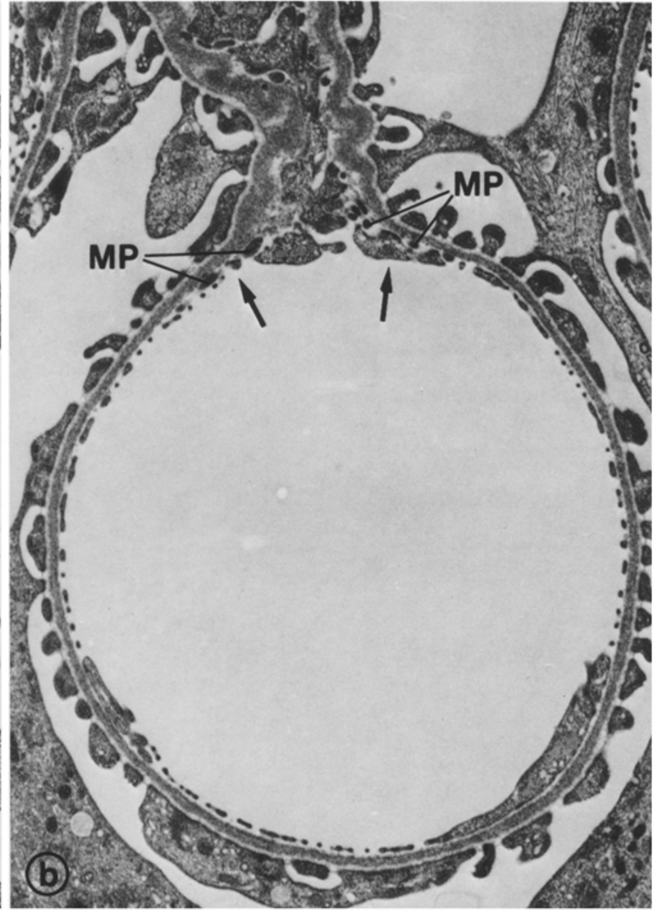
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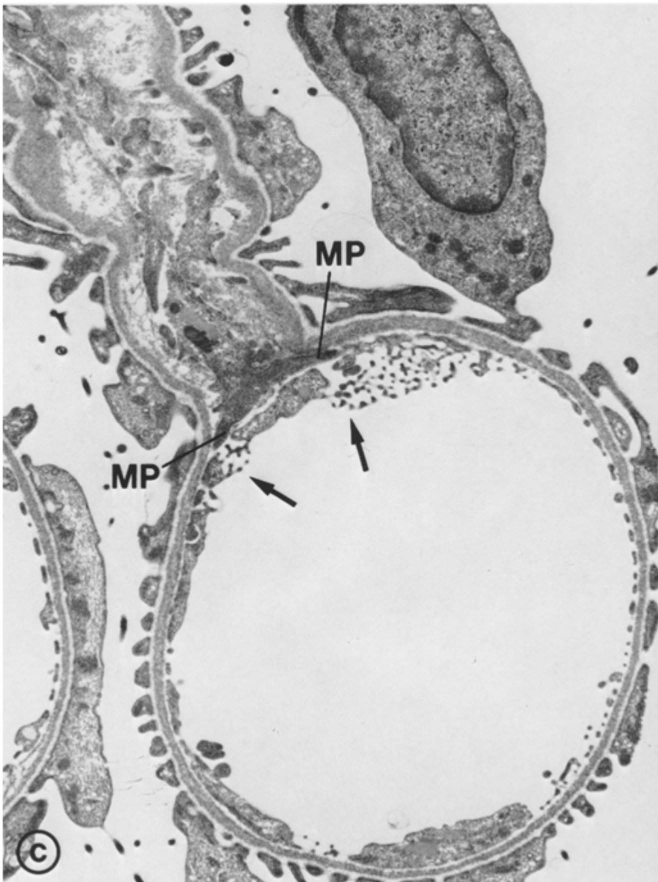
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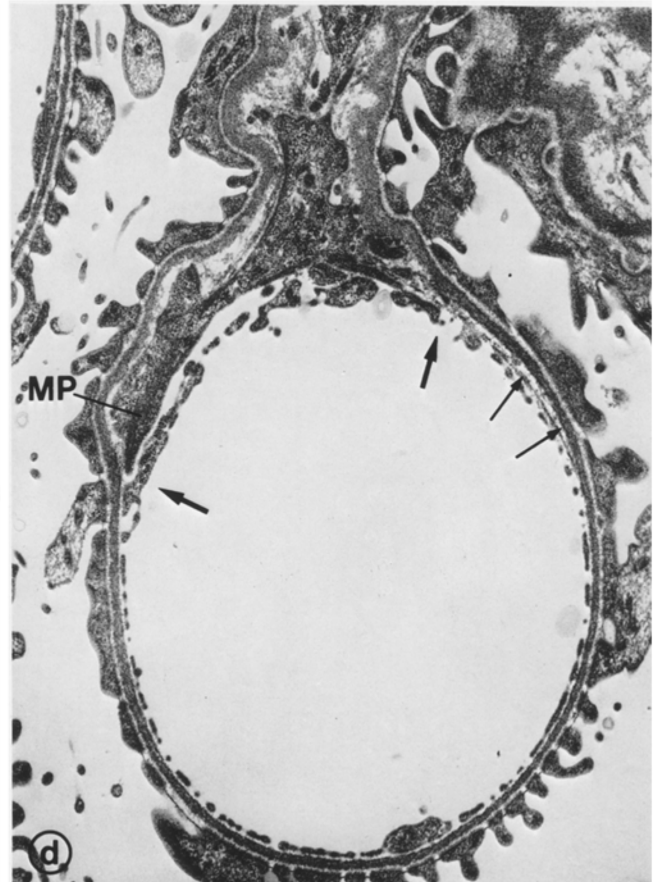
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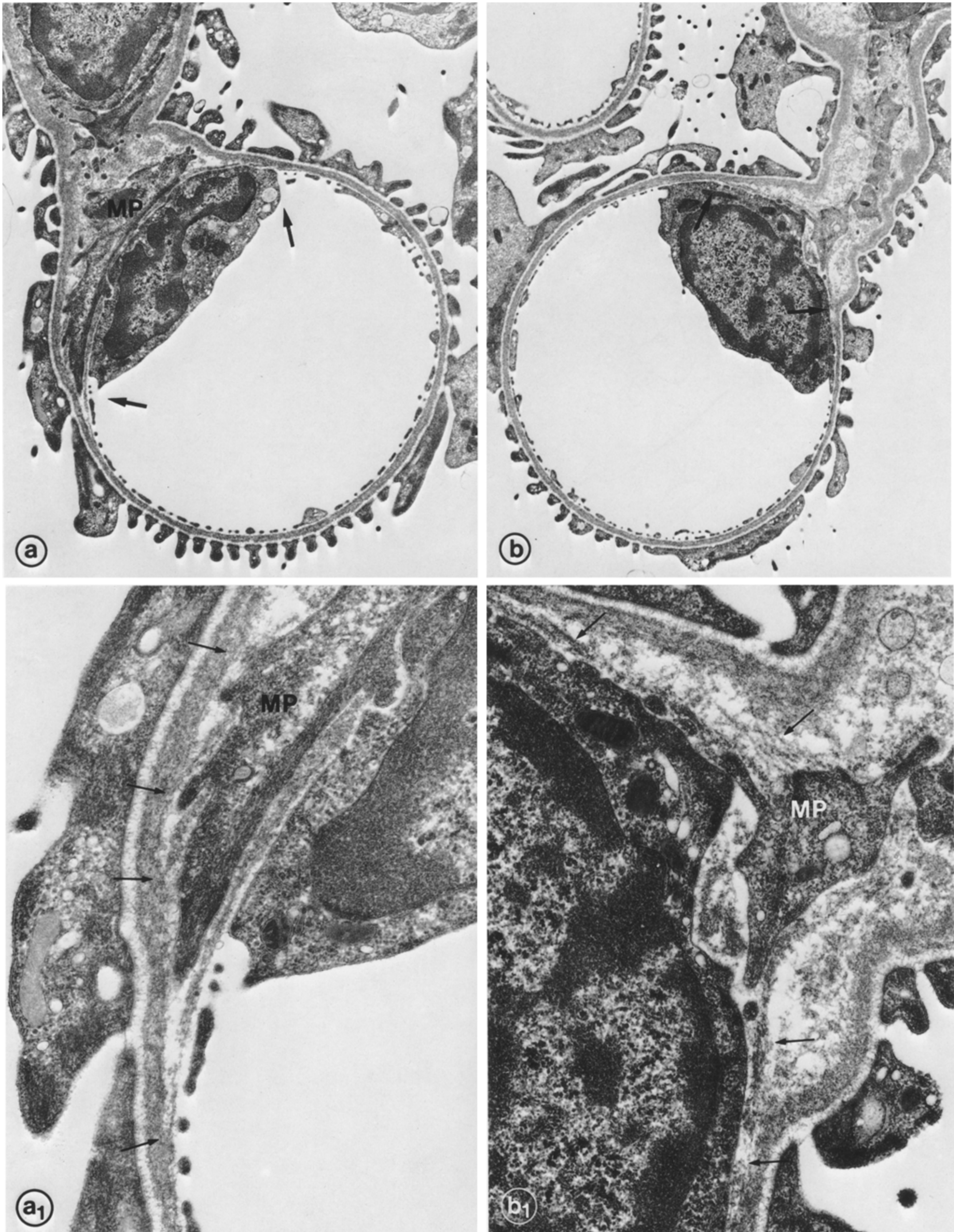

Fig. 2. Profiles of glomerular capillaries, in which the cell body of the endothelium is contained in its juxtamesangial portion. Mesangial angles are marked by *thick arrows* (in **a** and **b**) and are enlarged in $\mathbf{a}_1$ and $\mathbf{b}_1$. Note that the pericapillary and the perimesangial part of the GBM are different in thickness and the latter is partially wrinkled. The tongue-like mesangial cell process (MP in **a** and $\mathbf{a}_1$) has several indentations, out of which bundles of matrix fibrils emerge, running tangentially to the GBM where they insert (*thin arrows*). In $\mathbf{b}_1$, microfibrils either individually or in bundles extend from an intermediately positioned mesangial cell process (*MP*) to both mesangial angles (*thin arrows*). Pre-dehydration staining. **a** $\times \sim 11000$, $\mathbf{a}_1$ $\times \sim 47000$, **b** ~ 7500, $\mathbf{b}_1$ $\times \sim 30000$

smaller *juxtamesangial portion* which is apposed to the mesangium. The GBM and the layer of podocytes both have a *pericapillary part* (associated with the urinary portion of the capillary wall) and a *perimesangial part*. Consequently, the mesangium also has two subdivisions, one is in contact with the capillary endothelium (*juxtacapillary region*), the other with the GBM (*sub-GBM region*). The border between two subdivisions of any of the components all coincide, thereby prominent angles are established, which we call *mesangial angles* (Figs. 1, 2, 6).

The urinary portion of the endothelium and the pericapillary parts of the GBM and of the podocyte layer (being all three smoothly apposed to each other) constitute the filtration area. At this site, the endothelium, at least for its major part, is extremely attenuated and fenestrated, the podocyte layer is typically split up into foot processes and the GBM is of a fairly constant thickness in a given capillary profile. In contrast, the perimesangial part of the GBM is folded and wrinkled, and the lamina densa generally varies in thickness and is mostly thicker than the pericapillary part (Figs. 1, 2). The perimesangial part of the podocyte layer is often no longer smoothly opposed to the GBM; frequently it does not follow the folds of the GBM but can be described as to span in total the wrinkled area (Fig. 5).

The mesangium is composed of stellate like mesangial cells and a fibril-rich matrix (mesangial matrix). Mesangial cells are branched out into many cytoplasmic processes running into all directions (Figs. 1, 2, 5). Large processes can be distinguished from abundant microvilli-like microprocesses, which arise directly from the cell body or from major cell processes. All these processes contain abundant microfilaments, mostly arranged in bundles (Figs. 1a, c, d; 2a, a_1; 3a, b, e; 4c). In the cytoplasm of the cell body innumerable microtubules and intermediate filaments are encountered, running into all directions and penetrating into cell processes. In addition, the cell body contains some mitochondria, a Golgi apparatus and some profiles of ER. The mesangial matrix is found to be made up of a delicate fibrillar meshwork and some weakly stained material inbetween (see below). It is obvious that this fibrillar meshwork is intimately related and connected to mesangial cell membranes as well as to the GBM.

Connections between mesangial cells and the GBM

Mesangial cells and the GBM appear to be mechanically connected throughout the mesangial region. The most prominent connections are found at the mesangial angles and will be described first.

Mesangial angles and the juxtacapillary region of the mesangium regularly contain mesangial cell processes of various shapes (Figs. 1–3). Most frequent are tongue-like processes, which extend for variable distances between the endothelium and the GBM running strictly parallel to the GBM. They are intimately related to either the lamina densa directly or to the lamina rara interna of the GBM (Fig. 3). In other places the distance between cell processes and the lamina densa may be greater than the usual thickness of the lamina rara interna. In those cases fine fibrils are usually observed to run obliquely between the cell membrane and the lamina densa (Figs. 3, 4). Frequently, cell processes within two opposite mesangial angles originate from an interposing common trunk (Figs. 1, 3). Consequently, the space in the juxtacapillary region of the mesangium is completely bridged and the GBM from opposite mesangial angles is interconnected by a single mesangial cell process.

All these cell processes are filled with bundles of microfilaments which run in a tangential direction to the circular circumference of the capillaries (Figs. 1–3). These bundles appear to be anchored to the cytoskeleton in larger trunk processes, or to span the entire distance in the case of processes that extend from one mesangial angle to the opposite (Fig. 3).

A less frequent type of mesangial cell processes in mesangial angles are microvilli-like microprocesses. Like larger processes, they establish contact with the GBM, again either directly with the lamina densa, more frequently with the lamina rara interna or mediated by some interposed fibrillar structures (Figs. 3, 4).

In addition to direct or close attachments just described, mesangial cell processes which are remote from the GBM may be indirectly connected to the GBM at mesangial angles through bundles of extracellular fibrils (Figs. 3, 4). These connecting fibrils are straight, hollow structures of variable thickness representing microfibrils. They are mostly somewhat thicker than nearby cell membranes, but even much thicker fibrils may be encountered. They run in a tangential direction with respect to the capillary cylinder and insert within the GBM either just where the GBM deviates from its pericapillary course or more distally after running parallel to the pericapillary part of the GBM (Figs. 1, 2, 4). In favourable sections, it can clearly be noted that these extracellular fibrils continue the tangential direction of intracellular actin bundles (Figs. 2, 3).

This description of different kinds of mesangial cell-GBM connections at mesangial angles should not be taken as indicating the existence of various independent types. It is rather implied that the description relates to section profiles. All intermediates as well as various combinations are regularly encountered. Microprocesses may arise successively from a tongue-like process and run toward the GBM in angles that obviously adopt to a tangential course (Fig. 2a_1). From indentations inbetween those microprocesses, bundles of microfibrils emerge which also approach the GBM in a tangential direction. Thus various connection patterns between all kinds of cell processes and the GBM – directly or by close attachments and indirectly by microfibrils – are encountered (Fig. 6).

On the other hand mesangial angles are found, where obvious connections between the GBM and the mesangial cells are not detected in a given section. To estimate the incidences of connections, all mesangial angles of two glomerular profiles were photographed and evaluated. In total, 168 profiles of mesangial angles were analyzed. In 83 cases (=49.4%) direct connections between mesangial cell processes and the GBM, in 41 cases (=24.4%) connections mediated by microfibrils were encountered, amounting together to 73.8%. In all the other angles no obvious structural connections between mesangial cells and the GBM were seen.

Mechanical coupling of the GBM with mesangial cells is not restricted to mesangial angles; the entire perimesangial part of the GBM appears to be connected to mesangial cells (Fig. 5). One of the favourable features of our staining technique is that the mesangial matrix can be easily distinguished from the GBM. As mentioned above, a dense fibril-

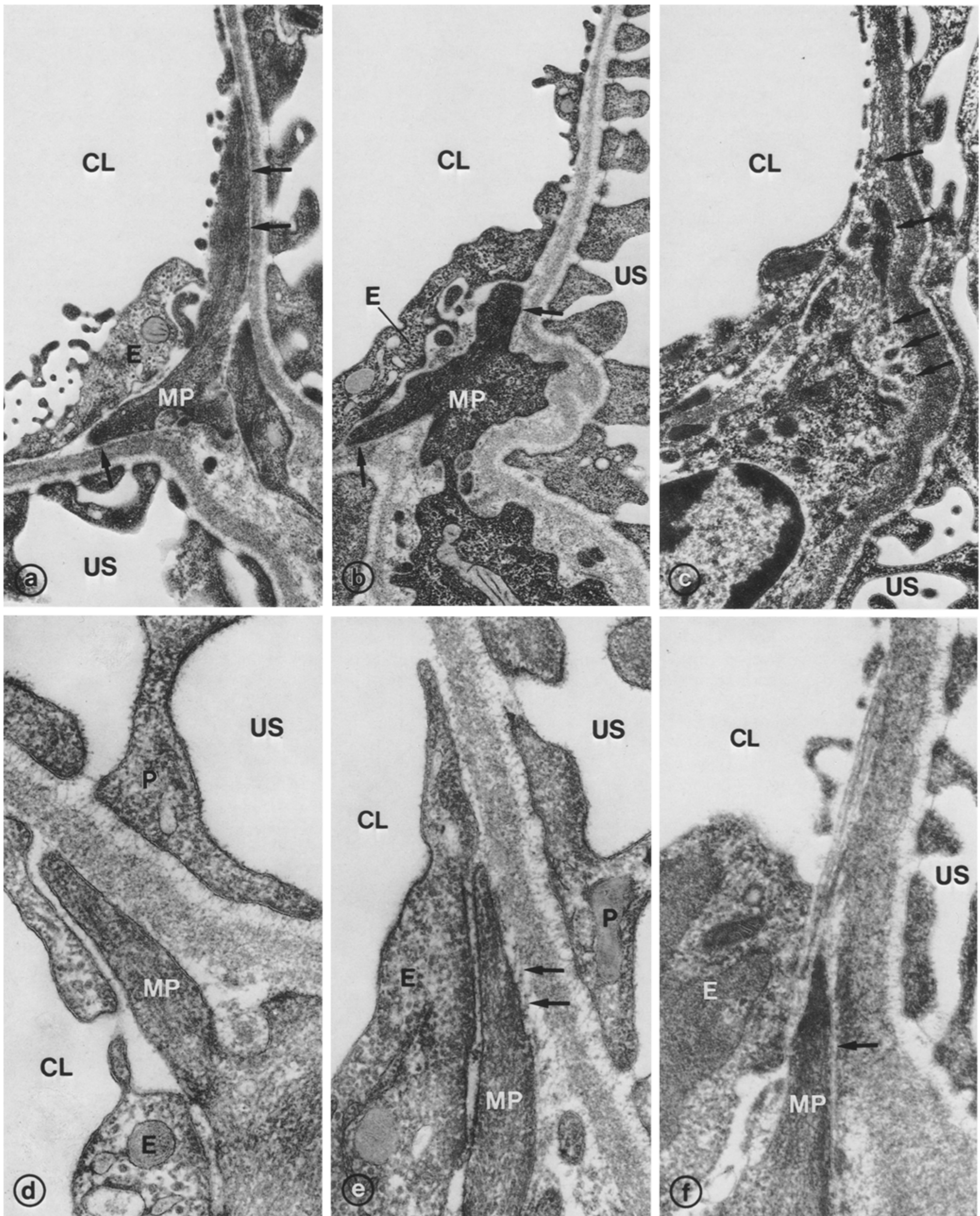

Fig. 3a–f. Profiles of mesangial angles to show various details of close attachments between mesangial cell processes (*MP*) and the *GBM*. **a** A mesangial cell process, which is filled with assemblies of actin filaments, bridges the entire distance between two opposing mesangial angles; at both sites it is closely attached to the GBM (*arrows*). Note the loose connection between the mesangial cell process and the endothelium. **b** A mesangial cell process, shorter than in **a**, bridges two opposing mesangial angles; the bridging portion is filled with tangentially running actin filaments. Note the coupling to the GBM at both mesangial angles and the loose connection between the mesangial cell process and the endothelium. **c** A mesangial angle which contains several profiles of mesangial

lar meshwork is found throughout the mesangial matrix which fills all the spaces between the perimesangial part of the GBM and the cell bodies and cell processes of mesangial cells. It has been described to be characterized "by the presence of small bundles of fine fibers embedded in its feltwork of finer fibrils" (Farquhar and Palade 1962). In our hands this fibrillar meshwork shows different textures depending on the staining technique employed: after pre-dehydration staining, it frequently appears to consist of individual hollow fibrils (microfibrils) which are heavily interwoven, but which do not branch (Figs. 4c, 5b). In contrast, after post-dehydration staining a more delicate texture of seemingly branching fibrils is observed (Figs. 4b, d). This meshwork is not homogeneously dense throughout. Bundles of fibrils are frequently accumulated near mesangial cells, often filling bay-like invaginations of mesangial cells (Fig. 5b). Microfibrils are easily recognized as hollow fibrillar structures after pre-dehydration staining, but are less obvious after post-dehydration staining.

It is by this fibrillar meshwork that the perimesangial part of the GBM appears to be completely fettered to mesangial cells. At sites where the GBM is heavily folded, the tops of folds pointing towards the mesangium are frequently found to be either directly related to mesangial cell processes or to be most intensively associated with fibrils of the mesangial matrix (Fig. 5b).

Relationships of the endothelium to the mesangium and to the GBM

Endothelial cells are composed of a cell body (containing the nucleus), which is surrounded by large attenuated and fenestrated cytoplasmic sheets. These sheets are much larger in area than the cell bodies. In about half of the profiles of glomerular capillaries an endothelial cell body is encountered which is mostly (but not necessarily) contained within the juxtamesangial portion of the endothelium (Fig. 2). The other half of capillary profiles does not show a cell body; in these cases, the juxtamesangial portion of the endothelium (like the urinary portion) is usually made up by fenestrated endothelial sheets or by peripheral parts of the cell body cytoplasm (Fig. 1).

The outer aspect of the juxtamesangial portion of the endothelium (irrespective of whether it belongs to a cell body or is fenestrated) is more or less directly related to mesangial cell processes. A basement membrane is not found, but wisps of mesangial matrix or bundles of matrix fibrils (running tangentially to the capillary wall) may be interposed between an endothelial cell and a mesangial cell. Mostly, the two cell types come closely together (Fig. 3), only separated by a narrow extracellular cleft, which may appear completely empty. Specialized intermembrane contacts were never encountered, even at those places where mesangial cell processes were found lying within cytoplasmic indentations of endothelial cells. Thus, in general, the linkage between mesangial and endothelial cells appears to be loose (Figs. 1, 3). Sometimes mesangial cell processes may be found to penetrate through a gap in the endothelium and to directly border the capillary lumen.

Discussion

Glomerular capillaries are a unique type of blood vessel. Their wall is made up of only one completely encircling layer, the endothelium, which, however, is for its most part extremely attenuated and fenestrated. These capillaries are perfused with a high hydraulic pressure resulting in a high transmural pressure difference. From its delicate structural organization the endothelium appears to be unable to develop a sufficiently high wall tension to match the distending forces created by the transmural pressure difference. The other components of the wall, the GBM and the layer of podocytes do not completely encircle the endothelial tube. Consequently, they cannot counteract the distending forces of the capillary wall alone. The structural completeness of these vessels (as of any other small vessels) will depend on a physical equilibrium between the distending forces caused by the transmural pressure and the wall tension developed by structural components of the vessel wall. The question which are the structures to develop and to maintain sufficient wall tension in these specific capillaries has never been adequately addressed.

Our findings provide evidence that the GBM and the contractile mesangial cells cooperate to evolve wall tension in glomerular capillaries. As has been shown by Burton (1951) the total tension in the wall of small vessels consists of two components, a passive component, due to all 'elastic' constituents in the vessel wall able to resist stretch by developing tension and an active component represented by contractile elements in the vessel wall. In the glomerular capillaries the active component appears to be represented by the mesangial cells, the passive component mainly by the GBM (surely, the endothelium, the mesangium and possibly also the layer of podocytes, will contribute an unknown but presumably small fraction).

The contractility of mesangial cells is not debated. Bundles of microfilaments (actin filaments) have been demonstrated in this and other studies (Becker 1972; Andrews and Coffey 1983). In addition, the contractility has been directly shown in many in vitro studies of cultured mesangial cells (Kreisberg 1983; Tanaka et al. 1984; Savin 1986; Singhal et al. 1986). Also the GBM appears appropriate for the supposed mechanical function. The highly compact meshwork of type IV collagen within the lamina densa is generally believed to impart a remarkable tensile strength to the GBM (Kanwar 1984; Timpl 1986). Thus, we propose that the elastic GBM and the contractile mesangial cells together establish a biomechanical unit that is capable of active tension development. From our findings it seems possible that contraction of mesangial cells should retract the GBM, and bring the GBM at opposing mesangial angles closer together. For this mechanism to operate, it is neces-

microprocesses (*arrows*); they appear to be connected to the GBM either directly or by fibrillar structures. **d** Mesangial angle with a tongue-like mesangial cell-processes, which appears to be directly apposed to the lamina densa of the GBM. **e** Mesangial angle with a tongue-like mesangial cell process which is connected to the GBM by short microfibrils (*arrows*). **f** Mesangial angle with a mesangial cell-process, which is in close contact to the GBM (*arrow*); in addition, three long microfibrils connect this process to more distant parts of the GBM. *CL*, capillary lumen; *US*, urinary space; *E*, endothelium; *P*, podocyte; **c** post-dehydration staining, all others pre-dehydration staining. **a** × ~23000; **b** × ~30000; **c** × ~24000; **d** × ~80000; **e** × ~54000; **f** × ~45000

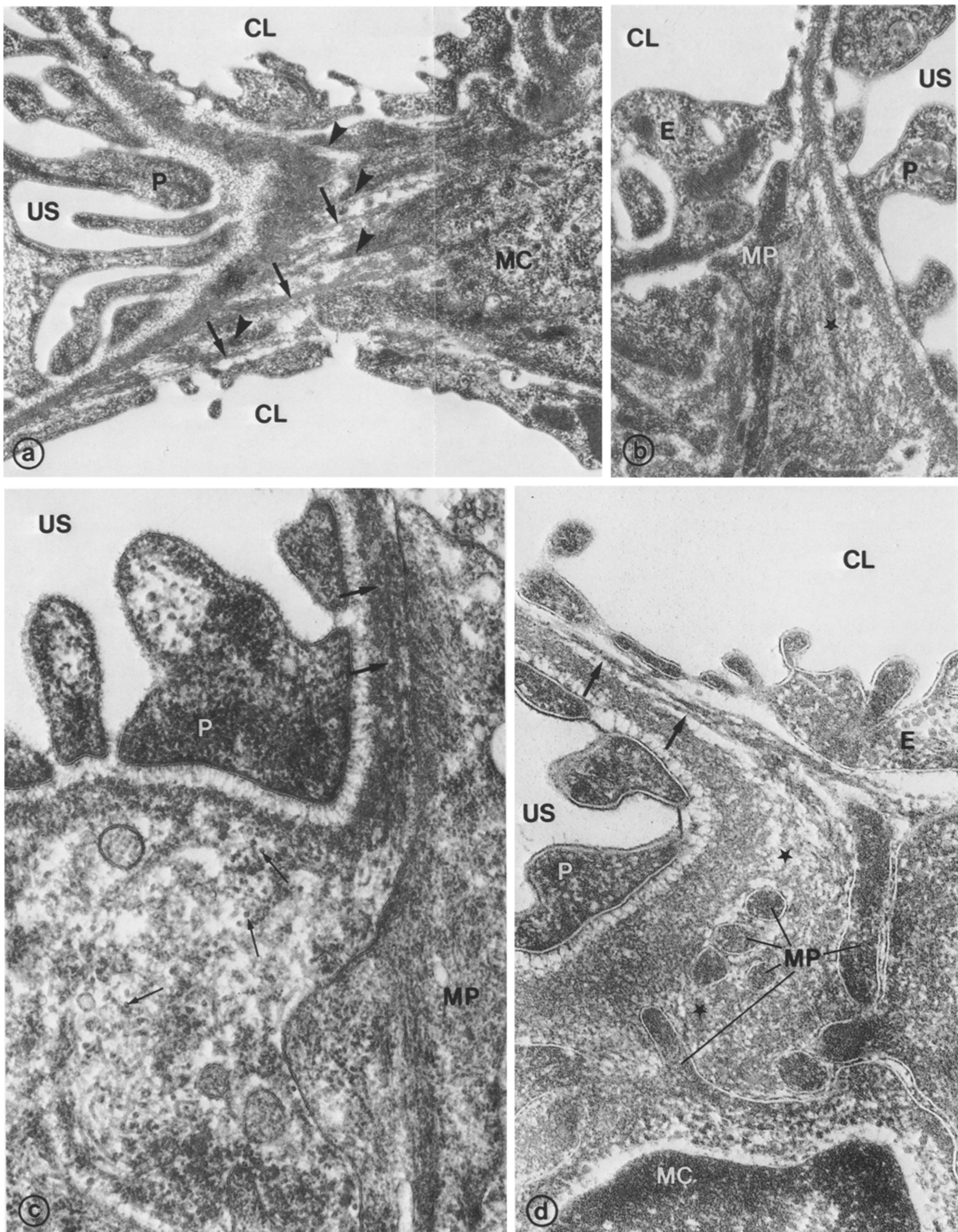

Fig. 4. Profiles of mesangial angles to show microfibrillar connections of mesangial cell processes and the GBM. **a** Grazing section through a mesangial angle showing bundles of matrix fibrils (*arrows*), which connect a mesangial cell (*MC*) with the GBM. The fibril bundles appear to alternate with mesangial cell processes (*arrow heads*). **b** At this mesangial angle the entire space between a mesangial cell process (*MP*) and the GBM appears to be filled by fibrillar material (*). **c** Mesangial angle with many microfibrils exhibiting, when cross-sectioned, a tubular structure (*thin arrows*). Note that these microfibrils are somewhat thicker than adjacent cell membranes.

sary that mesangial cells and the GBM are mechanically coupled with each other. Appropriate connections are predominantly necessary at mesangial angles. Therefore we have so far focused our study on these particular sites. However, mesangial cell – GBM connections exist throughout the mesangial region including the perimesangial parts of the GBM.

The mesangial cell – GBM connection at mesangial angles

In most profiles of mesangial angles, processes of mesangial cells are found. Without having performed a reconstruction, we have drawn a three-dimensional model of the presumptive relationships of the structures at mesangial angles (Fig. 7). We presume that a large mesangial cell process accompanies each capillary where the latter faces the mesangium. This process may be compared in its shape to a long one-legged console. The console plate has deep fissures running towards the leg (often including the leg), and gives rise to tongue-like processes extending towards and along the GBM at mesangial angles. These secondary processes will directly contact the GBM or will stay in some distance, in which case they are connected to the GBM by fibrils. The various profiles of mesangial cell process-GBM connections (as described above and summarized in Fig. 6) appear to result from random sections through those tongue-like processes. Profiles of mesangial angles without any mesangial cell processes may result from random sections through indentations. The typical tongue-like shape of mesangial cell processes and of their extensions into the space between the GBM and the endothelium has been frequently observed (Latta et al. 1960; Farquhar and Palade 1962; Helmchen 1980) but the widespread connections to the GBM were obscured in osmicated specimens.

Mesangial cell processes are coupled to the GBM at mesangial angles both by direct attachments and indirectly by microfibrils. The mesangial cell processes are consistently stuffed with bundles of actin filaments running tangentially to the cylindrical vessel in the direction of an anticipated stretch produced by the intracapillary pressure. In favourable sections it can clearly be seen that these actin bundles are continued outside the cells by microfibrils which finally anchor in the GBM.

The direct contact between mesangial cell processes and the GBM is intimate since a lamina rara is often lacking, and the cell membranes appear to be fixed to the lamina densa directly. In other cases a lamina rara is found, but in addition to its general composition some thicker strands are often seen bridging the distance between the cell membrane and the lamina densa. Fibronectin and laminin, in general, are essential to cell-matrix connections; both compounds have been identified at mesangial cell to matrix interfaces (Courtoy et al. 1980).

The indirect coupling is accomplished by microfibrils. The term microfibril designates a specific type of hollow, extracellular fibrils. They have been identified in the connecting tissue of many organs including the periphery of elastic fibers (Ross 1973; Goldfischer et al. 1983), muscle tendon junctions (Hanack and Böck 1971), insertions of muscles and subepithelial connective tissue (Demmel et al. 1979), Bruch's membrane of the eye (Essner and Gordon 1984) and ocular zonules of the ciliary body (Streten and Licary 1983; Inoue and Leblond 1986). Bundles of microfibrils are known as oxytalan fibres which have been found e.g. in the periodontol ligaments or in the aortic wall (Fullmer and Lilly 1958; Goldfischer et al. 1983; Takagi et al. 1984). At all these sites microfibrils are functionally interpreted as to establish a firm and somewhat elastic linkage between two structures; they appear to function as widespread anchoring filaments between cells and connective tissue fibers (Böck 1983; Goldfischer et al. 1985).

In the normal mammalian renal glomerulus tubular microfibrils have been found so far only in minute amounts in the GBM, in the subendothelial spaces and in the mesangial region (Courtoy et al. 1980; Farquhar 1981, Olesnicky et al. 1984). However, in certain renal diseases microfibrils accumulate within the GBM, mostly within the lamina rara interna and within the mesangial region (Hsu and Churg 1979; Yoshikawa et al. 1982; Olesnicky et al. 1984). In lower vertebrates including cyclostomes (Tsujii et al. 1984a, b), chondrichteyes (Bargmann and Hehn 1971) and amphibians (Sakai, unpublished) microfibrils are a regular occurrence in the subendothelial spaces of glomerular capillaries. By our modified staining technique we are able to clearly identify hollow microfibrillar structures as a usual constituent of the mesangial matrix in the rat.

From a recent thorough study of microfibrils in the eye and the foot pad of the mouse (Inoue and Leblond 1986), a tentative structural model of microfibrils has emerged. According to this study, the microfibril tubule is composed of successive annular segments possibly held together by a helically running surface band. Recently, two groups (Gibson et al. 1986; Sakai et al. 1986) have isolated two glycoproteins from microfibrils reporting molecular weights of 31 kD and 350 kD, respectively. Immunohistochemical evidence suggests that the amyloid P component is a major core constituent of microfibrils (Inoue et al. 1986) associated on its surface with fibronectin (Schwartz et al. 1985; Rostagno et al. 1986). This suggestion agrees with the finding that fibronectin is important for the anchoring of microfibrils to cell membranes (Goldfischer et al. 1985) or even across cell membranes to intracellular stress fibres (Singer 1982). In addition to a ubiquitous distribution within the mesangial matrix (Madri et al. 1980; Linder et al. 1980), fibronectin has specifically been found to decorate surfaces of mesangial matrix fibrils of about 10 nm thickness (Courtoy et al. 1980). Thus the abundant occurrence of fibronectin within the mesangial matrix correlates with the distribution of microfibrils. Both together are obviously involved in fixing the GBM to mesangial cells.

The thickness of microfibrils has been reported to vary between 4 and 35 nm, with figures from 11 and 15 nm being most frequently reported (Yoshikawa et al. 1982; Cleary and Gibson 1983; Olesnicky et al. 1984; Inoue and Leblond 1986). In our specimens microfibrils in the mesangial region are variable in thickness and were sometimes found to be

The mesangial cell process (*MP*), which contains assemblies of actin filaments, is very intimately related to the GBM (*thick arrows*). **d** Mesangial angle with several small mesangial cell processes (*MP*). They are connected to the GBM either by short fibrillar structures, which appear to form a network (*), or by long microfibrils (*arrows*) which accompany the GBM for some distance before they insert. *CL*, capillary lumen; *US*, urinary space; *E*, endothelium; *P*, podocyte; *MP*, mesangial cell process. **a, b,** and **d** post-dehydration, **c** pre-dehydration staining. **a** × ~26000, **b** × ~34000, **c** × ~65000, **d** × ~72000

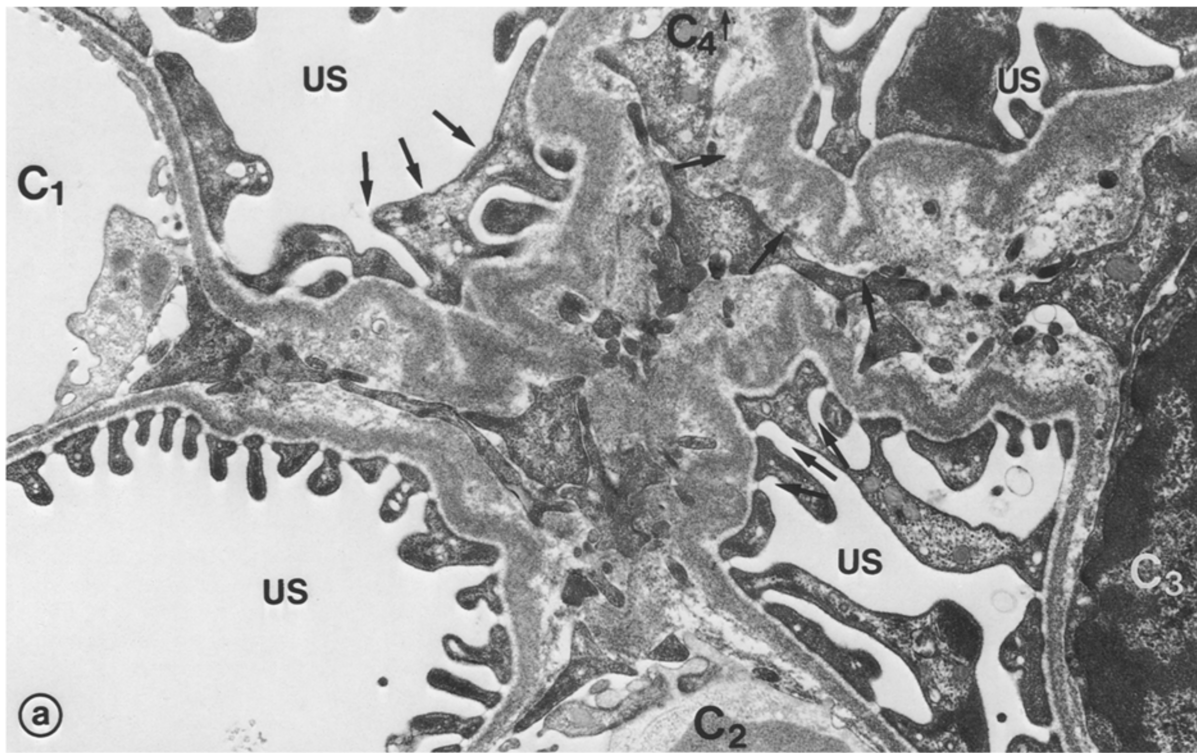

Fig. 5a–b. Centrolobular region of the mesangium. **a** cross-section through the centre of a glomerular lobule to which four capillaries are attached (C_1–C_4; C_4 lies fully outside the area which is shown); also the urinary space (*US*) is encountered four times. The perimesangial part of the GBM is heavily wrinkled. The perimesangial part of the podocyte layer does not always follow the folds of the GBM but spans in total the wrinkled area (*arrows*). **b** Part of a centrolobular region of the mesangium. It is bordered on

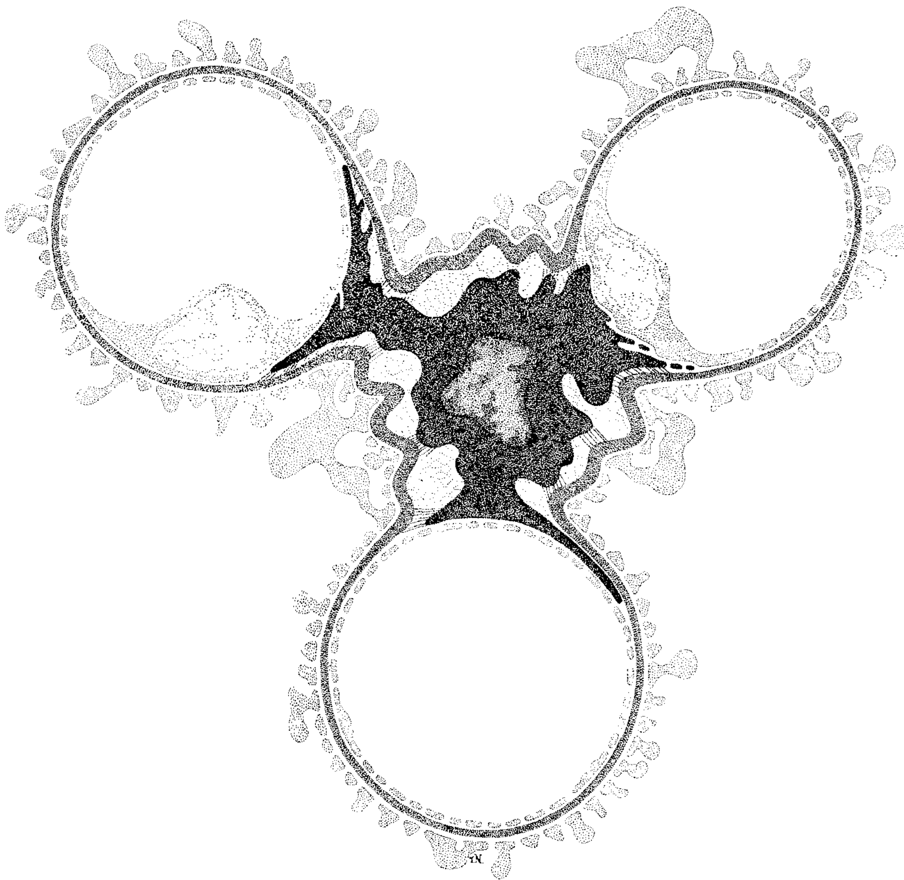

Fig. 6. Schematic representation of a cross-section through a glomerular lobule to summarize the main findings of this study. Three glomerular capillaries are attached to the centrally located mesangium. The podocytes and the endothelium are drawn in light grey, separated by the GBM, which is shown somewhat darker. The mesangial cell is drawn in dark grey. The mesangial matrix is shown in a fibrillar texture. Note the two subdivisions of either component: The endothelium consists of a urinary and a juxtamesangial portion. The GBM and the layer of podocytes are both composed of a pericapillary and a perimesangial part. Within the mesangium itself a sub-GBM region may be distinguished from a sub-endothelial region. Within the center of the mesangium, a mesangial cell is located, which gives rise to three major cell processes running towards the three capillaries. They split into secondary processes of various shapes, which fill the mesangial angles. Note that these processes are either closely attached to the GBM or connected to it by microfibrils. Additional connections between the mesangial cell and the GBM are found in the entire sub-GBM region of the mesangium

even thicker than cytoplasmic microtubules in adjacent cells. Attempts to further characterize the mesangial microfibrils with respect to thickness range, chemical composition and distribution within the mesangial matrix distant from mesangial angles are in progress.

In conclusion, we propose that the GBM – in addition to its role as a determinant of the filtration barrier – serves as the major skeletal structure of the glomerular tuft. Its firmness and tensile strength are the prerequisites that mesangial cells can insert to it, and thus contractions of mesangial cells can be transferred to the GBM and to the capillary wall. From a biomechanical viewpoint the GBM and mesangial cells should be considered as a functional unit which together balance the distending forces of the intracapillary pressure and thus maintain the structural integrity of glomerular capillaries.

The role of the glomerular endothelium

Our interpretation of the wall structure of glomerular capillaries does not give much weight to the endothelium as a component able to resist to stretch generated by the intracapillary pressure. Vasmant and coworkers (1984) have investigated the glomerular endothelium with respect to its armarment with cytoskeletal elements. They have found an organized cytoskeleton with some microtubules and intermediate size filaments in the cell body cytoplasm, and with primarily microfilaments (in addition to some microtubules) in the fenestrated area. These findings are confirmed by the present study and are in agreement with an immunocytochemical demonstration of vimentin in glomerular endothelial cells (Bachmann et al. 1983).

The function of these cytoskeleton elements may be mul-

both sides first by the perimesangial part of the podocyte layer (*P*), followed by the permesangial part of the GBM, which is heavily wrinkled. The centrally located mesangium contains a mesangial cell process (*MP*) giving rise to many microprocesses. The mesangial matrix, which can clearly be distinguished from the GBM, contains microfibrils, which are hollow in cross sections (*thin arrows*). In addition, some thicker homogeneous profiles of obviously cross-sectioned fibrils are encountered (*arrow heads*). Pre-dehydration staining. **a** × ~18000, **b** × ~57000

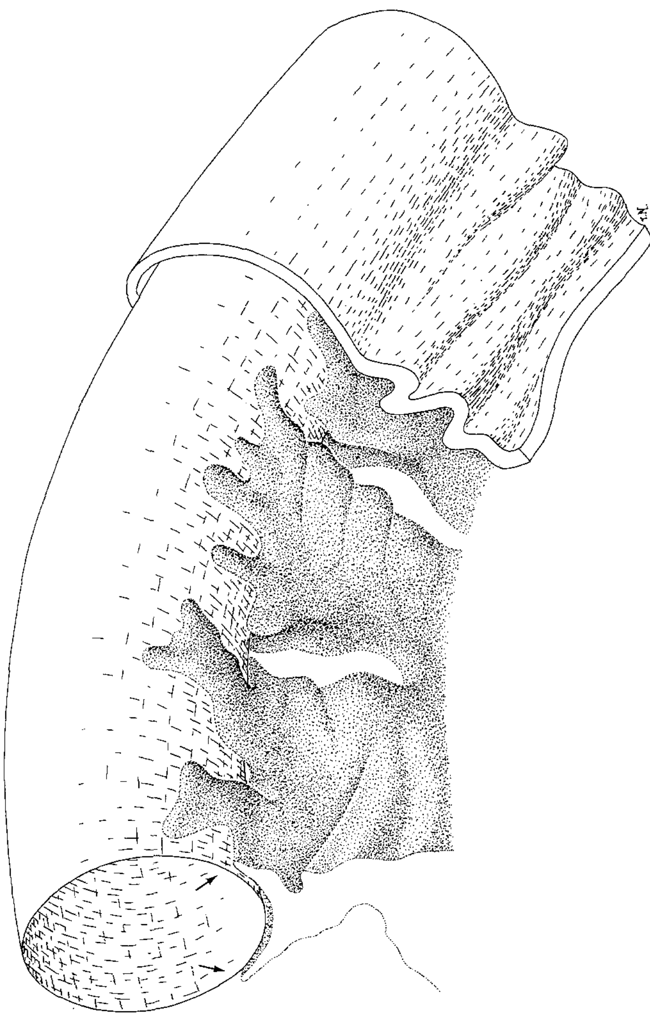

Fig. 7. Three-dimensional model of the relationships of a mesangial cell process to the endothelial tube of a glomerular capillary and to the GBM. The layer of podocytes is not drawn. The juxtamesangial portion of the endothelium (between the two arrows which indicate the mesangial angles) is underlaid by a mesangial cell process, which may be compared in its shape with the console. This process gives rise to many tongue-like secondary processes, which extend for various distances into the space between the endothelial tube and GBM. Note the transition from the smooth pericapillary part of the GBM to the wrinkled perimesangial part

tifold including to maintain the highly organized pattern of narrowly arranged fenestrations (suggested from their circular distribution around individual fenestrations; Vasmanti et al. 1984). For a role in opposing wall tension a preferentially tangential course of cytoskeleton elements would be required (as is established in circular smooth muscle layers for example). Such a pattern in the glomerular endothelium was not apparent, neither in this nor the previous study. Complete circular bundles of cytoskeleton elements are absent in the glomerular endothelium.

Endothelial cells could theoretically contribute to the mechanical stabilization of glomerular capillaries by a further mechanism. The cell bodies of endothelial cells are often contained within the juxtamesangial portion of the endothelium; thus they bridge the GBM from opposing mesangial angles and a role like that discussed above for mesangial cells might be assigned to them: Being attached to opposing parts of the GBM they might function as a mechanical link between them. Arguments against a major role of this kind again arises from the distribution of cytoskeleton elements (cytoskeleton bundles bridging opposing parts of the cell body cytoplasm are not apparent) and from the fact that the mesangial cell – GBM connections are not different whether the juxtamesangial portion of the endothelium is established by the thin fenestrated cytoplasm or by cell bodies. This latter observation may be considered as additional evidence that the mesangial cells are the appropriate structures to interconnect opposite parts of the GBM at mesangial angles. The relationships between mesangial cells and the basal aspect of the juxtamesangial portion of the endothelium (thus a type of a cell-to-cell interface) have been found to be remarkably loose. A narrow, sometimes wider intercellular space is encountered, which often is almost completely empty. It appears from our findings that mesangial cells and the endothelium are not at all fixed to each other. In contrast, by immunocytochemistry, the mesangial endothelial cell interface has been found to stain strongly for fibronectin (Courtoy et al. 1980). When comparing the staining patterns of that study with our pure structural findings, we would like to suggest that a strong reaction to fibronectin might result when microfibrillar bundles run tangentially from a mesangial cell to the GBM. Instances where bundles of microfibrils are found just beneath the juxtamesangial portion of the endothelium and adjacent to mesangial cell process are not rare and can easily be seen in several figures (Figs. 1a, 2b, 3b). At higher magnification, it can clearly be identified that the fibril bundles at this site do not connect mesangial and endothelial cells but are orientated tangentially to the basal aspect of the endothelium. The relevance of the seemingly loose apposition of the juxtamesangial portion of the endothelium to the underlying mesangium remains to be established.

Functional considerations

It has been shown by many studies (for original literature see Dworkin and Brenner 1985; Brenner and co-workers 1986) that mesangial cell contraction exerts considerable effects on glomerular filtration dynamics. Modulation of mesangial contractility by hormones may change intraglomerular blood flow and filtration characteristics under physiological and pathological conditions. However, the link between mesangial cell contraction and alterations in glomerular hemodynamics, i.e. experimental findings about the geometrical changes within the glomerular tuft going along with mesangial cell contractions have not been presented so far. In vivo-observations have revealed a change in intraglomerular blood flow distribution in response to AII stimulation with a relative shift of blood flow from long to short pathways through the glomerular capillary net (Steinhausen 1986), but the underlying mechanism is unknown. Constriction of selective capillaries by mesangial cell contraction could be the appropriate cause, but a model of how mesangial cell contraction leads to constriction of individual capillaries is lacking. The present study provides the structural basis to think in geometrical terms about the consequences of mesangial cell contraction and relaxation.

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References

Andrews PM, Coffey AK (1983) Cytoplasmic contractile elements in glomerular cells. Fed Proc 42:3046–3052

Ausiello DA, Kreisberg JJ, Roy C, Karnovsky MJ (1980) Contraction of cultured rat glomerular mesangial cells after stimulation with angiotensin II and arginine vasopressin. J Clin Invest 65:754–760

Bachmann S, Kriz W, Kuhn C, Franke WW (1983) Differentiation of cell types in the mammalian kidney by immunofluorescence microscopy using antibodies to intermediate filament proteins and desmoplakins. Histochem 77:365–394

Bargmann W, Hehn GV (1971) Über das Nephron der Elasmobranchier. Z Zellforsch 114:1–21

Becker CG (1972) Demonstration of actomyosin in mesangial cells of the renal glomerulus. Am J Pathol 66:97–110

Böck P (1983) Elastic fiber microfibrils: Filaments that anchor the epithelium of the epiglottis. Arch Histol Jpn 46:307–314

Brenner BM, Dworkin LD, Ichikawa I (1986) Glomerular ultrafiltration. In: Brenner BM, Rector FC (eds) The kidney, 3rd edn. Saunders, Berlin, vol 1, pp 124–144

Bretschneider HJ (1980) Myocardial protection. Thorac Cardiovasc Surg 28:295–302

Burton AC (1951) On the physical equilibrium of small blood vessels. Am J Physiol 164:319–329

Carlemalm E, Garavito RM, Villinger W (1982) Resin development for electron microscopy and an analysis of embedding at low temperature. J Microsc 126:123–143

Cleary EG, Gibson MA (1983) Elastin-associated microfibrils and microfibrillar proteins. Int Rev Connect Tissue Res 10: 97–209

Courtoy PJ, Kanwar YS, Hynes RO, Farquhar MG (1980) Fibronectin localization in the rat glomerulus. J Cell Biol 87:691–696

Demmel U, Schewe U, Böck P, Gorgas K (1979) Die Feinstruktur der Muskel-, Sehnen- und Muskel-Epithelverbindungen in der Zunge des Meerschweinchens. Cytobiol 18:460–477

Dworkin LD, Brenner BM (1985) Biophysical basis of glomerular filtration. In: Seldin DW, Giebisch G (eds) The kidney: physiology and pathophysiology, Raven Press, New York, pp 397–426

Essner E, Gordon SR (1984) Demonstration of microfibrils in Bruch's membrane of the eye. Tissue Cell 16:779–788

Farquhar MG (1981) The glomerular basement membrane – a selective macromolecular filter. In: Hay ED (ed) Cell biology of extracellular matrix. Plenum Press, New York, pp 335–378

Farquhar MG, Palade GE (1962) Functional evidence for the existence of a third cell type in the renal glomerulus. Phagocytosis of filtration residues by a distinctive "third" cell. J Cell Biol 13:55–87

Foidart JB, Mahieu P (1986) Glomerular mesangial cell contractility in vitro is controlled by an angiotensin-prostaglandin balance. Mol Cell Endocrinol 47:163–173

Fullmer HM, Lillie RD (1958) The oxytalan fiber: a previously undescribed connective tissue fiber. J Histochem Cytochem 6:425–430

Gibson MA, Hughes JL, Fanning JC, Cleary EG (1986) The major antigen of elastin-associated microfibrils is a 31-kDa glycoprotein. J Biol Chem 261:11429–11436

Goldfischer S, Coltoff-Schiller B, Goldfisher M (1985) Microfibrils, elastic anchoring components of the extracellular matrix, are associated with fibronectin in the zonule of zinn and aorta. Tissue Cell 17:441–450

Goldfischer S, Coltoff-Schiller B, Schwarz E, Blumenfeld OO (1983) Ultrastructure and staining properties of aortic microfibrils (oxytalan). J Histochem Cytochem 31:382–390

Goldman RD, Chojnackl B, Yerna MJ (1979) Ultrastructure of microfilament bundles in baby hamster kidney (BHK-21) cells. J Cell Biol 80:759–766

Hanak H, Böck P (1971) Die Feinstruktur der Muskel-Sehnenverbindung von Skelett- und Herzmuskel. J Ultrastruct Res 36:68–85

Helmchen UE (1980) Die Zahl der Mesangiumzellen in einem normalen Glomerulum der Rattenniere. Eine dreidimensionale elektronenoptische Analyse. Med Diss, Tübingen

Hsu H-C, Churg J (1979) Glomerular microfibrils in renal diseases: A comparative electron microscopic study. Kidney Int 16:497–504

Inoue S, Leblond CP (1986) The microfibrils of connective tissue: I. Ultrastructure. Am J Anat 176:121–138

Inoue S, Leblond CP, Grant DS, Rico P (1986) The microfibrils of connective tissue: II. Immunohistochemical detection of the amyloid P component. Am J Anat 176:139–152

Kaissling B, Kriz W (1982) Variability of intercellular spaces between macula densa cells: a transmission electron microscopic study in rabbits and rats. Kidney Int 22 [Suppl 12]:9–17

Kallerhoff M, Blech M, Kehrer G, Kleinert H, Siekmann W, Helmchen U, Bretschneider HJ (1986) Post-ischemic renal function after kidney protection with the HTK-solution of Bretschneider. Urol Res 14:271–277

Kanwar YS (1984) Biology of Disease. Biophysiology of glomerular filtration and proteinuria. Lab Invest 51:7–21

Kreisberg JI (1983) Contractile properties of the glomerular mesangium. Fed Proc 42:3053–3057

Kühn K, Sterzel RB, Stolte H, Reale E (1976) Mesangial cells in different vertebrate kidneys. A thin-section and freeze-fracture study. In: Sterzel RB, Thomson D, Brod J (eds) Contrib Nephrol. Karger, Basel, vol 2, pp 9–16

LaFountain JR, Zobel CR, Thomas HR, Galbreath C (1977) Fixation and Staining of F-Actin and Microfilaments using tannic acid. J Ultrastruct Res 58:78–86

Latta H, Maunsbach AB, Madden SC (1960) The centrolobular region of the renal glomerulus studied by electron microscopy. J Ultrastruct Res 4:455–472

Laurie GW, Leblond CP, Inoue S, Martin GR, Chung (1984) Fine structure of the glomerular basement membrane and immunolocalization of five basement membrane components to the lamina densa (Basal lamina) and its extensions in both glomeruli and tubules of the rat kidney. Am J Anat 169:463–481

Linder E, Miettinen A, Törnroth T (1980) Fibronectin as a marker for the glomerular mesangium in immunohistology of kidney biopsies. Lab Invest 42:70–75

Madri JA, Roll FJ, Furthmayr H, Foidart JM (1980) Ultrastructural localization of fibronectin and laminin in the basement membranes of the murine kidney. J Cell Biol 86:682–687

Maupin P, Pollard TD (1983) Improved preservation and staining of HeLa cell actin filaments, clathrin-coated membranes, and other cytoplasmic structures by tannic acid-glutaraldehyde-saponin fixation. J Cell Biol 96:51–62

Olesnicky L, Doty SB, Bertani T, Pirani CL (1984) Tubular microfibrils in the glomeruli of membraneous nephropathy. Arch Pathol Lab Med 108:902–905

Pricam C, Humbert F, Perrelet A, Orci L (1974) Gap junctions in mesangial and lacis cells. J Cell Biol 63:349–354

Ross R (1973) The elastic fiber. A review. J Histochem Cytochem 21:199–208

Rostagno A, Frangione B, Pearlstein E, Garcia-Pardo A (1986) Fibronectin binds to amyloid P component. Localization of the binding site to the 31,000 Dalton C-terminal domain. Biochem Biophys Res Commun 140:12–20

Sakai LY, Keene DR, Engvall E (1986) Fibrillin, a new 350-kD glycoprotein is a component of extracellular microfibrils. J Cell Biol 103:2499–2509

Sakai T, Sabanovic S, Hosser H, Kriz W (1986) Heterogeneity of the podocyte membrane in rat kidney as revealed by ethanol dehydration of unosmicated specimens. Cell Tissue Res 246:145–151

Savin VJ (1986) In vitro effects of angiotensin II on glomerular function. Am J Physiol 251:F627–F634

Schnabel PhA, Richter J, Gebhard MM, Pomykaj Th, Preusse CJ, Ulbricht LJ, Bretschneider HJ (1985) Comparison of fixation by immersion and by perfusion after cardioplegia. Verh Anat Ges 79:311–314

Schwartz E, Goldfischer S, Coltoff-Schiller B, Blumenfeld OO (1985) Extracellular matrix microfibrils are composed of core proteins coated with fibronectin. J Histochem Cytochem 33:268–274

Simionescu N, Simionescu M (1976) Galloylglucoses of low molecular weight as mordant in electron microscopy. I. Procedure, and evidence for mordanting effect. J Cell Biol 70:608–621

Singhal PC, Scharschmidt LA, Gibbons N, Hays RM (1986) Contraction and relaxation of cultured mesangial cells on a silicone rubber surface. Kidney Int 30:862–873

Singer II (1982) Association of fibronectin and vinculin with focal contacts and stress fibers in stationary hamster fibroblasts. J Cell Biol 92:398–408

Skorecki KL, Ballermann BJ, Rennke HG, Brenner BM (1983) Angiotensin II receptor regulation in isolated renal glomeruli. Fed Proc 42:3064–3070

Sraer JD, Sraer J, Ardaillou R, Mimoune D (1974) Evidence for renal glomerular receptors for angiotensin II. Kidney Int 6:241–246

Steinhausen M, Kücherer H, Parekh N, Snoei H (1986) Microcirculation in glomerular network: Implication of hemodynamic factors in effect of angiotensin II on glomerular function. In: Popel, Johnson (eds) Microvascular Networks. Experimental and Theoretical Studies. S. Karger, Basel, 1986, pp 134–141

Streeten BW, Licari PA (1983) The zonules and the elastic microfibrillar system in the ciliary body. Invest Ophthalmol Vis Sci 24:667–681

Takagi M, Parmley RT, Yagasaki H, Toda Y (1984) Ultrastructural cytochemistry of oxytalan fibers in the periodontal ligament and microfibrils in the aorta with the periodic acid-thiocarbohydrazide-silver proteinate method. J Oral Pathol 13:671–678

Tanaka T, Fujiwara Y, Orita Y, Sasaki E, Kitamura H, Abe H (1985) The functional characteristics of cultured rat mesangial cells. Jpn Circ J 48:1017–1029

Timpl R (1986) Recent advances in the biochemistry of glomerular basement membrane. Kidney Int 30:293–298

Tsujii T, Naito I, Ukita S, Ono T, Seno S (1984a) The anionic barrier system in the mesonephric renal glomerulus of the arctic lamprey, Entospheus japonicus (Martens) (Cyclostomi). Cell Tissue Res 235:491–496

Tsujii T, Naito I, Ukita S, Ono T, Seno S (1984b) The anionic barrier system in the mesonephric renal glomerulus of the brown hagfish, Paramyxine atami Dean (Cyclostomi). Anat Rec 208:337–347

Vasmant D, Maurice M, Feldmann G (1984) Cytoskeleton ultrastructure of podocytes and glomerular endothelial cells in man and in the rat. Anat Rec 210:17–24

Yoshikawa N, Cameron AH, White RHR, Standring DM (1982) Microfibrils in glomerulopathies of children: an ultrastructural study. J Pathol 136:123–131

Zimmermann KW (1929) Über den Bau des Glomerulus der menschlichen Niere. Z mikr anat Forsch 18:520–552

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