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Basement membrane changes in atrophic tubules in the human kidney

Gerda Goovaerts[1], **Marc E. De Broe**[2], **and Norbert Buyssens**[1]

Departments of [1]Pathology and [2]Nephrology and Hypertension, University Hospital Antwerp, University of Antwerp, U.I.A. Wilrijkstraat 10, B-5220 Edegem-Antwerpen, Belgium

Summary. Changes in the basement membrane (BM) in atrophic tubules in human kidney biopsies were studied by electron microscopy and by immunohistochemistry on cryostat sections with antibodies against collagen type I, type III, type IV, laminin, EMA, keratin and vimentin.

The BM showed different degrees of thickening with formation of reduplications which contained fibrocytes. Remnants of cytoplasm of epithelial cells and fibrocytes were incorporated in the thickened BM. This showed signs of lysis and disintegration, indicating that the redundant BM formed by the epithelial cells is removed, although imperfectly, by interstitial cells. Thinning of the BM was another frequent finding. Immunohistochemistry showed a clear reactivity for collagen type IV and laminin in all BM material. The epithelial cells showed multilayering and a peculiar type of dark cells extending underneath adjacent cells and separating them from their BM attachment.

Key words: Kidney – Tubulus – Atrophy – Basement membrane

Introduction

Shrinking of renal tubules is generally accompanied by changes in the basement membrane (BM). The most frequent features are folding, wrinkling and thickening. Sometimes the BM is thinned or not detectable by PAS and methenamine silver stains. A peculiar change is reduplication with interposition of cells between the newly formed layers. The question of the nature of these cells arises: do epithelial cells become detached and left behind between the newly formed BM layers during the shrinking process, or do interstitial connective tissue cells populate the space which is left by the retracting epithelial tube? A further question pertains to the fate of the redundant BM material and to changes in immunoreactivity in its redundant state.

Offprint requests to: N. Buyssens

Materials and methods

Kidney biopsies from 20 patients showing severe tubular atrophy and BM lesions on light microscopy (LM) were selected for this study. The original kidney diseases comprised diabetic glomerulosclerosis, ischaemia, chronic glomerulonephritis, analgesic abuse, congenital hypoplasia and rejection after transplantation. Two apparently normal kidneys of patients with "benign" microscopic haematuria were used as controls. For LM the fragments were fixed in Duboscq-Brasil fixative, processed in paraffin and stained with the PAS and the methenamine silver stain of Jones.

Immunohistochemistry was performed on frozen material in 6 cases which were selected for the size of the tissue cylinder and for the severity of the atrophic lesions. The following antibodies were used: (1) Keratin: polyclonal antiserum raised in the rabbit against purified keratin of epidermal callus of human foot (Organon Technika); (2) Collagen type I: polyclonal antiserum raised in the rabbit against purified bovine collagen type I (Sodichimic); (3) Collagen type III: polyclonal antiserum raised in the goat against purified bovine collagen type III (Sodichimic); (4) Collagen type IV: polyclonal antiserum raised in the rabbit against human collagen type IV obtained by pepsin digestion of human placenta (Sodichimic); (5) Laminin: polyclonal antiserum raised in the rabbit against mouse laminin extracted from the E.H.S. tumour purified by immunoabsorption (Sodichimic); (6) Epithelial membrane antigen (EMA): monoclonal antibody against defatted human milk cream (Dako); (7) Vimentin: polyclonal antiserum raised in the rabbit against vimentin isolated from calf lenses by preparative gel electrophoresis (Organon Technika); (8) Desmin: polyclonal antiserum raised in the rabbit against chicken gizzard desmin, isolated by preparative gel electrophoresis (Organon Technika). The antibodies were applied on cryostat sections and visualized by the PAP method using DAB for detection.

Fragments for electron microscopy were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer and postfixed in 1% OsO_4 in veronal-acetate buffer. The fragments were block stained

with 2% uranyl acetate in veronal-acetate buffer and embedded in Epon 812. Ultrathin sections were examined with a Zeiss EM 109.

Results

The thickness of the BM in electronphotomicrographs in kidneys with normal tubules is rather variable but varies between 300–1000 nm. These figures are of course influenced by the section plane which could not be directed in biopsy cylinders. They correspond well with the figures obtained by Vracko et al. (1980).

The lesions are similar in all cases, but more pronounced in diabetic and ischaemic kidneys. They are schematically represented in Fig. 1 and in an overview in Fig. 2.

On reduplication of BM the thickened membrane divides and the two layers rejoin. They enclose spindle cells with many ramifications, much RER and indented nuclei with much heterochromatin (Figs. 3, 4). Sometimes they show condensations of filaments under the plasma membrane, preferentially at the side directed to the inner layer of the BM. The space between the cells and the BM contains many collagen fibers which often penetrate the thickened BM (Fig. 5). The cytoplasm of the cells shows extensions in the thickened BM which may reach up to the level of the original BM (Fig. 5). In many places the outer split membrane does not join the inner one and the space in between communicates freely with the interstitium containing fibrocytes, lymphocytes and sometimes smooth muscle cells or intermediate forms (myofibroblasts). This outer membrane ends free in the interstitium and often forms multiple branches which extend haphazardly (Fig. 4). Many isolated distorted fragments of thickened BM intermingled with collagen fibers and cells are found in the widened intertubular space.

Thickening of the BM may present in many different forms. The lamina lucida may be preserved, sometimes only in part of the tubule. Its presence is not related to the total thickness or to the structure of the BM. The lamina densa may be homogenous and in direct contact with the base of the epithelial cells. It may also be laminated, consisting of membranes of the same or of different width. Sometimes homogenous and laminated forms merge.

Fragments of collagen fibers are often incorporated into the thickened BM. They sometimes are located close to the epithelial cells at the place were they could be expected to lie if the BM were not thickened. If the thickened BM is composed of laminae with a wider interstitium, the latter contains many collagen fibers.

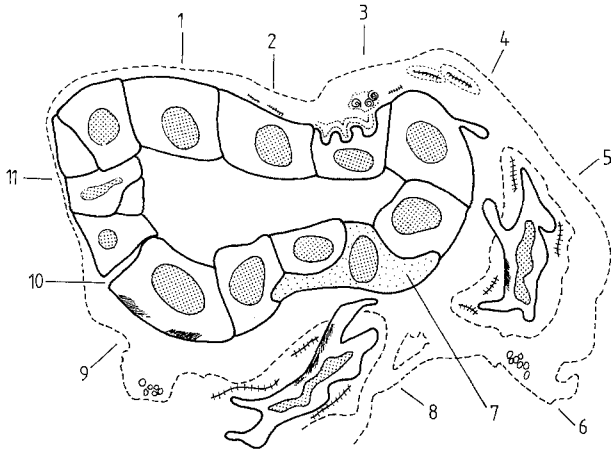
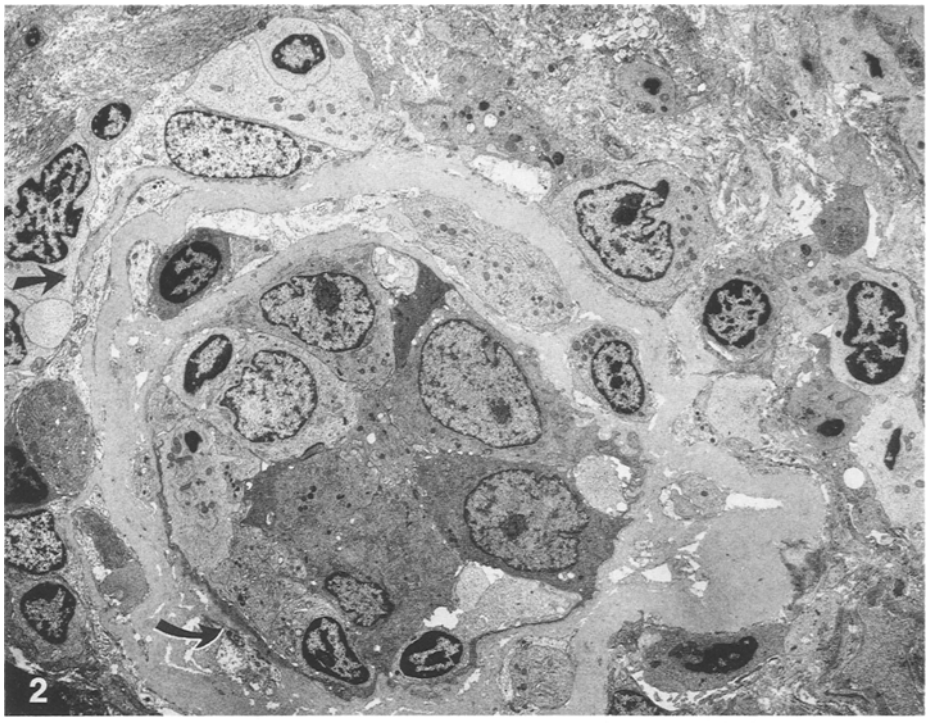


Fig. 1. *1.* Normal epithelial cell with BM of normal thickness. *2.* Collagen fibers close to the epithelial cell incorporated in the thickened BM. *3.* Retraction of basal part of epithelial cells with newly formed thin laminae of BM. In the thickened BM cytoplasmic remnants. *4.* Incorporated collagen fibers in broad laminae of BM. Extension of epithelial cell in BM. *5.* Closed reduplication of BM. Fibrocyte with extension in BM and increased number of filaments in the cytoplasm directed to the inner BM. Collagen fibers in pericellular space. *6.* Punched out defect at outer side of BM. Irregular contour of BM due to disappearance of the original thickened BM. *7.* Dark epithelial cell extending under adjacent cells and lifting them from the BM attachment zone. *8.* Open reduplication of BM. Holes in thickened BM, moth-eaten appearance. Extension of interstitial fibrocyte in BM. Many collagen fibers in the pericellular space. *9.* Epithelial cell with condensations of microfilaments. Punched out holes in BM and moth-eaten appearance of thickened BM. *10.* Intercellular BM. *11.* Multilayering of epithelial cells. Thinning of BM

A constant feature of the thickened BM is the presence of groups of rounded structures bound by a single or a double membrane and mixed with electron dense granules resembling ribosomes. These structures have the morphology of cell organelles and generally lie closest to the epithelial cells. Sometimes it is possible to demonstrate connections with the cytoplasm of extensions of epithelial (Figs. 6, 7) or of interstitial cells (fibrocytes). Another type of inclusion consists of rounded, sharply limited punched out holes, also lying in groups but generally at the outer side or even in apparently isolated clumps of BM in the interstitium. They bear no resemblance to cell organelles (Fig. 3).

The outer part of the thickened BM, the reduplications and the free ending extensions in the interstitium often show irregular borders or larger holes resulting in a moth-eaten appearance. Generally fibrocytes are present in the vicinity and one sometimes in contact with these BM fragments (Fig. 4).



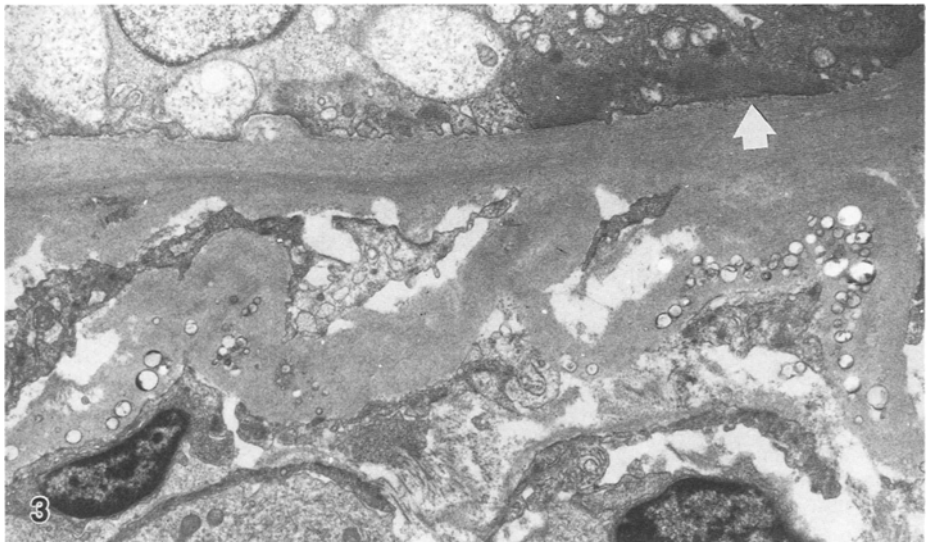


Fig. 2. Overview illustrating many of the features of BM changes: thickening, reduplication, free ending extension (*long arrow*), moth-eaten appearance. Presence of clear and dark epithelial cells, the latter with long extensions underneath adjacent cells (*curved arrow*). ×3300

Fig. 3. Reduplication of BM with interposition of fibrocytes. Punched out holes in the outer membrane. Moth-eaten appearance. Filament condensations at the base of epithelial cells (*arrow*). ×9500

The fibrocytes are always surrounded by preserved collagen fibers but are not invested by a basal lamina.

BM of adjacent tubules may lie in very close apposition and sometimes give the impression of being fused. The thickened BM around capillary pericytes may become incorporated in the thickened tubular BM.

The lamina densa of the BM may become very thin so that it is formed by a lamina lucida and densa which together are 50 to 75 nm thick (Figs. 8, 9). When the cytoplasm of the epithelial cells retracts a new thin BM may follow the cell membrane, or the original one may bridge the gap. Sometimes several layers of thin BM fill the gap or surround extensions of the cytoplasm which still remains at its original site (Fig. 10). Thinning is found in some tubules with apparent normal epithelial cells. In shrunken tubules thin segments may alternate with thickened areas. Exceptionally the BM cannot be recognized and damaged epithelial cells with signs of apoptosis are in direct contact with collagen fibers.

Identification of the type of epithelial cell is

Fig. 4. Reduplication and free ending fragment of thickened BM. Moth-eaten appearance. Many fibrocytes with long ramifications surrounded by collagen fibers. Filament condensations in fibrocyte cytoplasm. Cytoplasmic remnants in slightly laminated BM (*arrow*). Epithelial cell at bottom. ×9500

only possible when the organelles are well preserved which is not the case in atrophic tubules. Hence no correlation between defined segments of the tubules and BM changes could be made. There is, however, no constant relationship between the severity of BM changes and those of the epithelial cells.

The thickened BM may form crestlike nodular extensions between the lateral cell borders which come close to junctional complexes at the luminal side. Another type of BM extension is formed by rather thin intercellular strands of BM which partly surround cells and join the thickened BM (Fig. 11).

Some tubules are lined by a mixture of dark cells and clear cells. The dark cells often send long extensions underneath the clear cells and occupy the original contact zone between the clear epithelial cells and the BM (Figs. 2, 7). The electron density of the cytoplasm is caused by the presence of many microfilaments 7–9 nm thick. These filaments are also present in many other cells were they form bundles which are located at the basal pole. Sometimes they form strands which traverse the cytoplasm.

Many tubules show transections of superposed epithelial cells in the same plane, which indicates a process of multilayering. Epithelial cells of tubules with a small or a virtual lumen may have very long junctional complexes forming a radiating network.

Sometimes tubules containing only one cell are

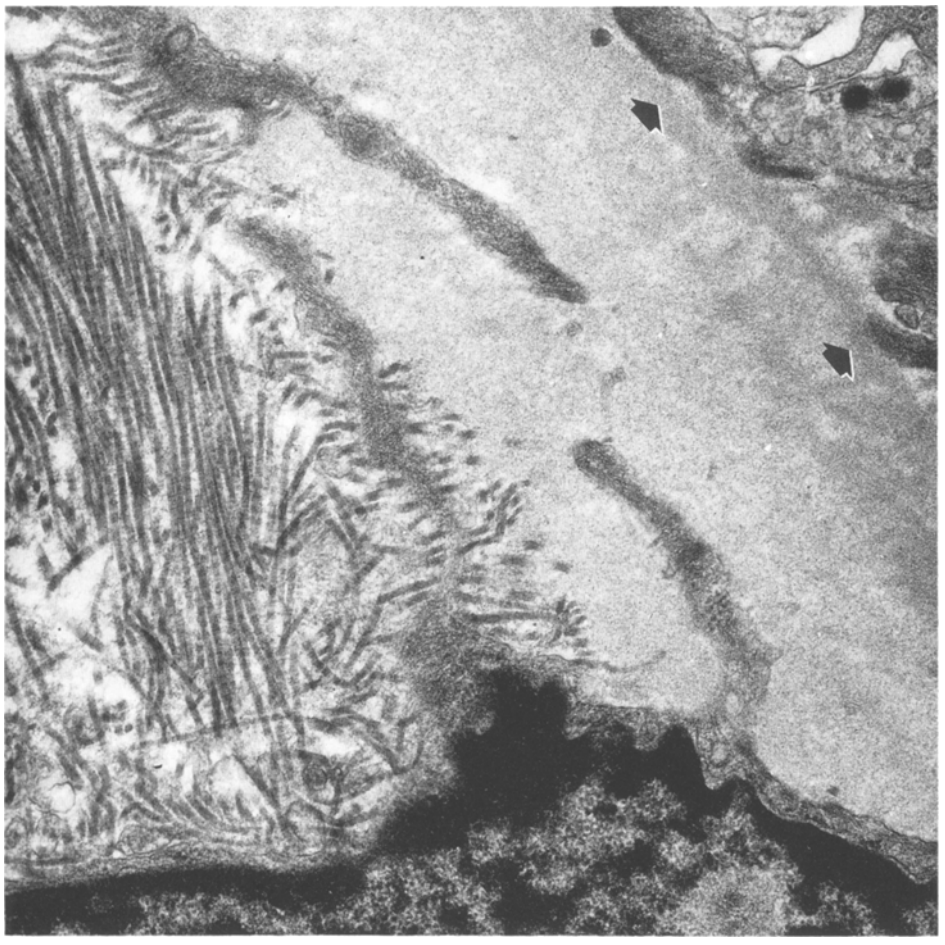


Fig. 5. Extensions of fibrocyte in thickened BM. Collagen fibers penetrating in the BM. Filament condensations at the base of epithelial cells (*arrows*). ×40000

found: the BM is either very thin or thickened. It is not possible to establish whether these epithelial cells are still in connection with the tubules or whether they are totally isolated and represent remnants of a tubule which has disappeared.

The immunohistochemical findings were consistent in the six cases. Three cases contained glumeruli. In EMA staining moderate positivity was present in parietal epithelial cells of the glomerulus; strong positivity was found in distal convoluted tubules and weak to strong positivity in atrophic tubules; proximal convoluted tubules showed no reactivity, nor did cells outside the BM.

Moderate positivity for keratin was present in parietal epithelial cells of the glomerulus; strong positivity was found in distal convoluted and collecting tubules, weak positivity appeared in some proximal convoluted tubules, atrophic tubules showed strong positivity often as a linear deposit close to the cell membrane; cells outside the BM were negative.

For vimentin moderate to strong positivity was present in podocytes of the glomerular tufts: a weak positivity within the tufts could not be assigned to mesangial or endothelial cells; strong positivity was found in all atrophic tubules and moderate positivity in isolated cells or in segments of epithelium of tubules with a wider lumen, lined by cylindrical or cuboidal cells and with a slight to moderate thickening of the BM. The positivity was sometimes limited to a linear deposit under the cell membrane at the apical pole. Desquamated epithelial cells lying free in the widened tubular lumina were also positive; positivity was clear in cells in the reduplicated BM, in interstitial cells and endothelial cells of capillaries and arterioles.

For collagen type IV moderate positivity was present in the BM of the glomerulus and of the Bowman capsule; moderate positivity was also demonstrated as a fine linear deposit at the base of the epithelium of normal tubules. The slightest thickening of the BM produced a strongly positive inner and outer line and a weaker positivity of the core. Only when the membrane was very thick, as in severe atrophy, was a distinct strong homogeneous positivity present (Fig. 12). In case of redu-

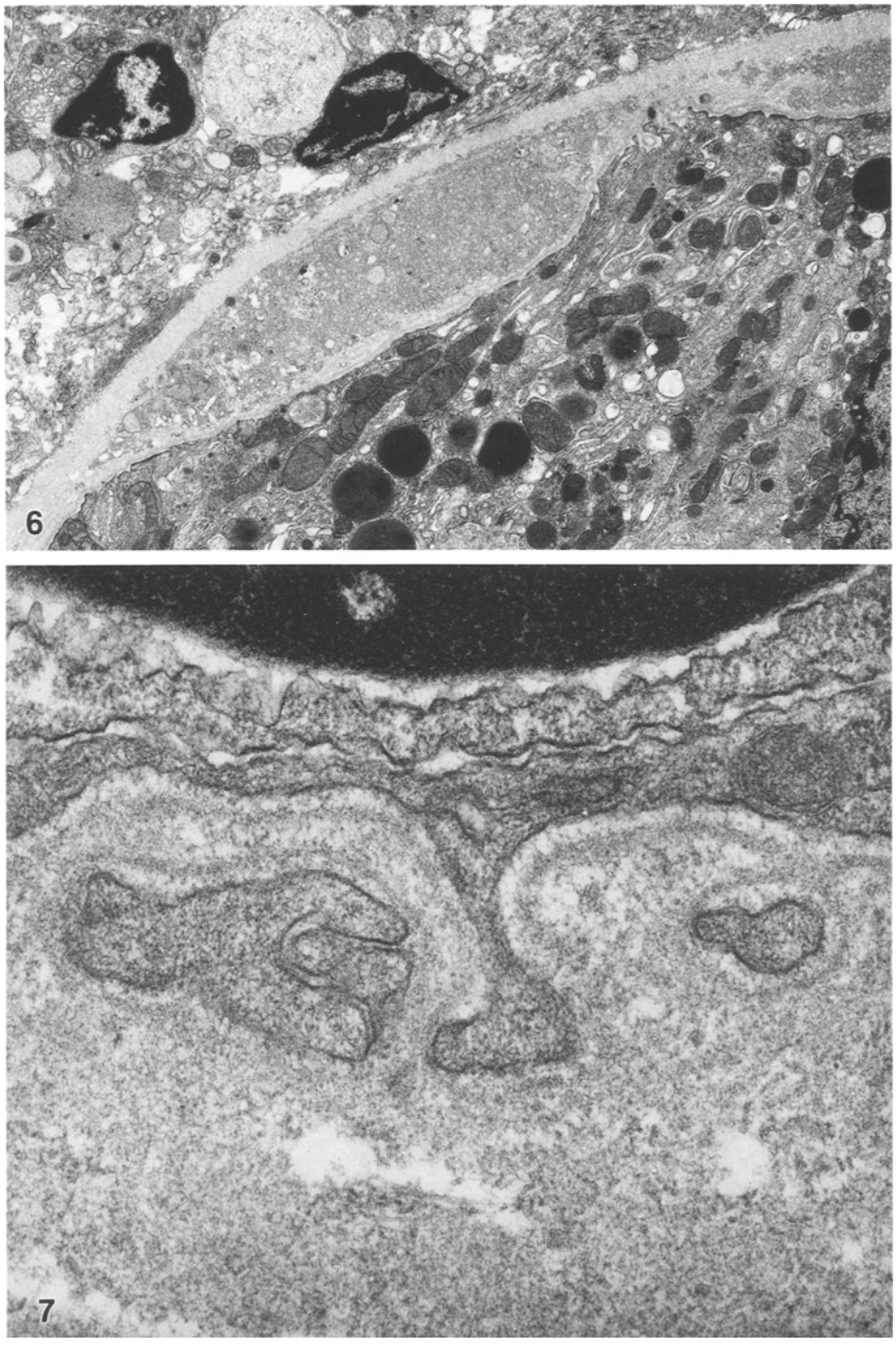


Fig. 6. Incorporation of cytoplasmic remnants into a reduplicated BM. Notice that the two membranes are thinner than normal. ×9500

Fig. 7. High power micrograph showing how cytoplasmic extensions of a dark cell remain in the layered thickened BM. Notice the intercellular space between the dark cell and the other epithelial cell on top. ×6600

plications of the BM the same well defined positive band lined the space surrounded by the doubled BM (Fig. 13). All the BM material, whether part of a tubule or lying free in the interstitium, showed the same reactivity.

In general reactivity for laminin was weaker than with collagen type IV but the diffuse staining of the core between the two limiting lines was better visible.

With collagen type I and type III antibodies moderate to strong positivity was present in the interstitial fibers. No reactivity with BM could be demonstrated.

In summary the epithelial cells of atrophic tubules express EMA, keratin and vimentin. Thickened BM expresses collagen type IV and laminin as strongly positive lines at each side of the membrane and show a diffuse staining of the core which

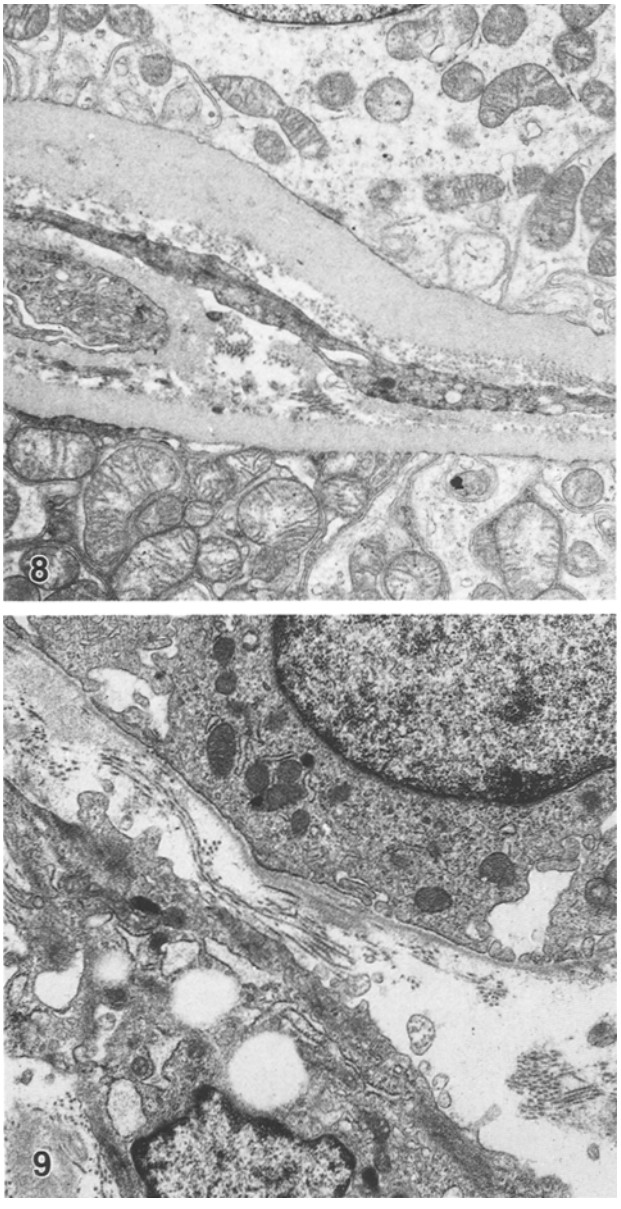


Fig. 8. Two tubuli, one with a normal BM, the other with a slightly thickened BM. ×9500

Fig. 9. Tubulus with an extremely thinned BM. Notice the close proximity of collagen fibers to the thinned BM. Same magnification as Fig. 9. ×9500

increases with the width of the BM. The reactivity is present in all the BM material in whatever localization.

Discussion

The cells lying between reduplicated BM present the morphological features of fibrocytes and are invariably surrounded by collagen fibers. There are many reduplications open on one side and in free communication with the interstitial fibrocytes. No special mechanism is needed to explain their presence in the closed reduplications which are probably only closed over a limited distance. The positive reactivity for vimentin and the negative for cytokeratin confirm the E.M. findings. All BM material retains the immunoreactivity for collagen type IV and laminin.

The mechanism for the reduplication consists in a primary deposition of thickened BM, followed by retraction of the epithelial cells and formation of a new, first thin and later thickened BM. The stromal fibrocytes occupy the space between both BM sheats. It is surprising that redundant BM survives while lying free in the interstitium and that it retains its morphology as well as its immunoreactivity. The presence of interepithelial BM seems not to contribute to the development of the reduplicated BM. It is however an unexpected finding because epithelial cell polarity is normally not associated with BM formation at the lateral borders. Moreover in one case the cells were well preserved and showed normal microvilli. The laminated structure of the thickened BM and the presence of inclusions of cytoplasmic origin have been described in man (Flume et al. 1963; Vracko et al. 1980; Møller et al. 1984) and in the rat (Romen and Mäder-Kruse 1978). We demonstrated the continuity of cytoplasmic extensions of both epithelial cells and fibrocytes with these inclusions. Another type of inclusion, formed by rounded punched out defects was found especially in BM fragments away from epithelial cells: these fragments showed a moth-eaten appearance and were in the vicinity of fibrocytes surrounded by well preserved collagen fibers. These features are consistent with a process of disintegration of BM occurring in the interstitium, while collagen fibers are preserved. It is interesting to mention that similar punched out defects are frequently found in the BM of Bowman's capsule. The presence of intact collagen fibers in a thickened BM at the place where they originally lie demonstrates that they become incorporated during the process of BM thickening. Other authors have concluded that BM formation can be considered to be a function of epithelial cells and of fibrocytes alike, although epithelial cells are the main producers. Our findings indicate that a lytic action of fibrocytes is the most likely cause of this appearance.

In embryological development of the nephron the mesenchymal cells which will become epithelial cells express extracellular matrix components before acquisition of an epithelial form: this indicates that BM formation and epithelial differentiation are linked (Saxen 1987). Moreover we could not

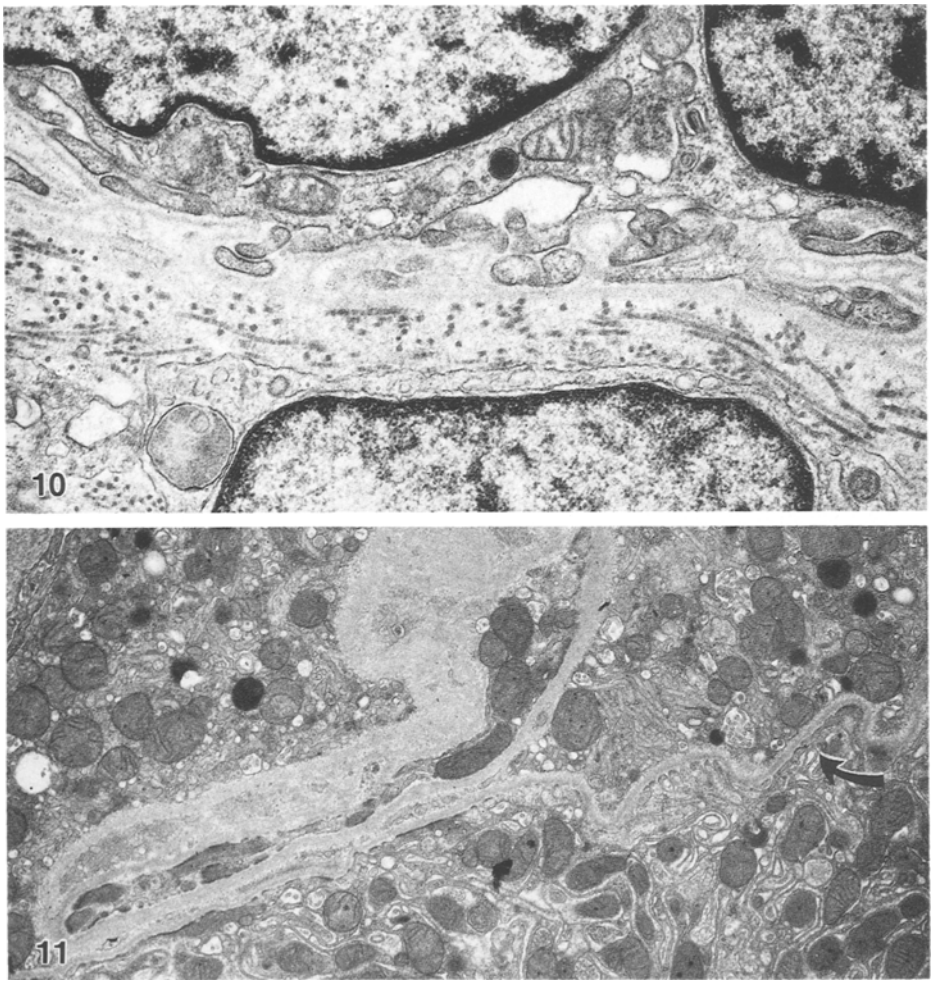


Fig. 10. Retraction of an epithelial cell with extensions left behind and invested by a multilayered BM composed of thin laminae. Notice the close proximity of collagen fibers. ×23000

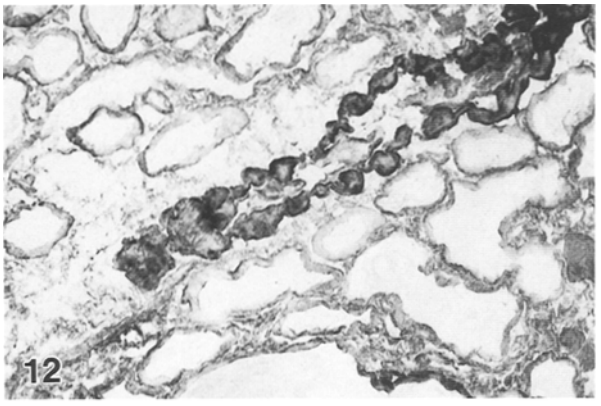
Fig. 11. Cytoplasm of four epithelial cells showing intercellular BM of different thickness. Notice the very thin undulating segment (*arrow*). ×9500

find a basal lamina surrounding fibrocytes. There are myofibroblasts and smooth muscle cells invested by a thin basal lamina in the interstitium, but they are away from zones with tubular BM formation.

A feature which has not attracted much attention is the thinning of the BM. Zollinger et al. (1973) illustrate and describe it in ischaemic contracted kidneys. Romen and Mäder-Kruse (1978) mention the formation of a thin new BM when epithelial cells detach from the original BM. In our material thinning was found in many places and was not associated with retraction of epithelial cells. At light microscopy epithelial cells are often seen which are not surrounded by a BM stained by PAS or methenamine silver stains. At EM these cells are nevertheless surrounded by an extremely thin BM, less than 50 nm thick, which probably is too thin to be visible at LM. Why BM are so thin cannot be explained: suffice it to say that well preserved collagen fibers lie very close and that there are no features of disintegration.

The results obtained by immunohistochemistry confirm the immunoreactivity of all BM for collagen type IV and laminin. Frozen material was used to eliminate fixation artefacts and because most immunohistochemical reactions are stronger and more consistent in cryostat sections. We were surprised to find that immunoreactivity is the same in BM material of different thickness, form or age. This preservation of immunoreactivity is in agreement with the work of Abrahmson and Caulfield (1982) who found that in the glomerular basement membrane anti-laminin antibodies remain bound for at least several months. A striking feature was the heavier staining at the surfaces of slightly or severely thickened BM leading to a double contoured membrane. It may be a staining artefact or it may indicate a better availability of immunoreactivity sites at the surface of the membranes.

Lesions of epithelial cells have been studied in detail by Flume et al. (1963) and Møller et al. (1984). We can confirm these findings. Some additional features not reported by these authors are



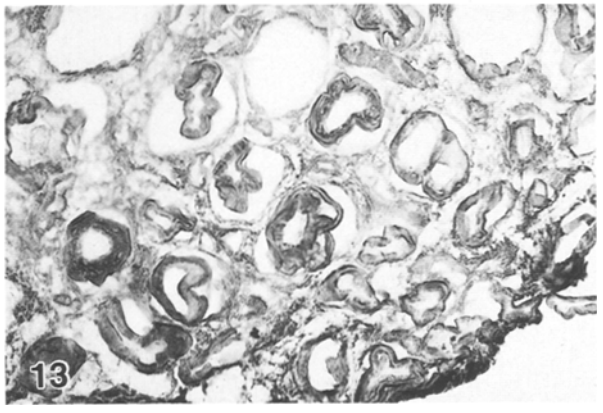


Fig. 12. Immunostaining for collagen type IV. A very thickened BM shows homogenous staining. ×230

Fig. 13. Immunostaining for collagen type IV. In the center a tubulus with two reduplications. The irregularity of the thickened membranes is a shrinkage artifact of the frozen tissue. ×230

interesting. First the presence of dark cells which contain many microfilaments and which extend underneath the adjacent cells, sometimes under more than one. These cells show a certain morphological resemblance to the intercalated cells in collecting ducts: they differ by their increased electron density which is due to an increase in microfilaments, and by their "undermining" behaviour. Second, the multilayering of epithelial cells which occurs often, but not exclusively at the end of a tubule, which is the place were it bends: this multilayering is the expression of a rearrangement of the normally regularly aligned cylindrical or cuboidal epithelial cells. Third, the presence of apparently isolated cells surrounded by a thin or thick BM: these may be section artefacts, but if considered spatially these cells must lie in a cup of BM. Some tubules are seen with a very small lumen which are composed of well preserved cylindrical or cuboidal cells with a small number of microvilli. Larger tubules contain scattered damaged cells with signs of apoptosis as described by Gobe and Axelsen (1987) in the rat.

Immunohistochemistry of the epithelial cells in atrophic tubules shows a clear reactivity for keratin which is stronger than in preserved tubules. The coexpression of vimentin confirms the findings of Gröne et al. (1987). Individual vimentin positive cells in apparently well preserved tubules are seen and similar cells are found lying free in the lumen: possibly one of the first changes of damaged epithelial cell may be the acquisition of vimentin positive intermediate filaments.

We conclude that the cells in the reduplicated BM are fibrocytes. Epithelial cells produce BM in excess and this BM is disintegrated in the interstitium. All the BM material conserves its immunoreactivity for collagen type IV and laminin. Atrophic tubules may be invested by a thinned BM. Epithelial cells may become multilayered and a special type of dark cell lifts adjacent cells from the BM.

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